# METHODS OF

# ORGANIC ANALYSIS

BY

HENRY C. SHERMAN, PH.D.

ADJUNCT PROFESSOR OF ANALYTICAL CHEMISTRY
IN
COLUMBIA UNIVERSITY

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# PREFACE.

The purpose of this work is to give a connected introductory training in organic analysis, especially as applied to plant and animal substances and their manufactured products. No attempt is made to touch upon all important branches of this subject but representative topics are treated in considerable detail with reference both to analytical methods and to the interpretation of results.

The greater part of the book is devoted to quantitative methods for food materials and related substances. Standard works of reference and the publications of the Association of Official Agricultural Chemists have been freely used. The nomenclature adopted in these publications has been followed as closely as possible. As a rule, footnotes show the original sources of statements or methods included in the text, while general or additional references are given at the end of each chapter. The references have been carefully selected and are believed to be sufficient to put the reader in touch with the most important literature.

The descriptions of methods were written primarily for the use of third-year students in the School of Chemistry, Columbia University, and therefore presuppose a knowledge of inorganic quantitative analysis, elementary organic chemistry, and general physics.

The writer takes pleasure in acknowledging his indebtedness to Professor Edmund H. Miller for helpful advice and suggestions throughout the work, and to Mr. Roland H. Williams for assistance in testing methods and in the revision of parts of the manuscript.

H. C. S.

NEW YORK, July 1, 1905.

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# CHAPTER I.

## Introduction.

### ULTIMATE AND PROXIMATE ANALYSIS.

*Ultimate organic analysis* is the determination of the elements composing any organic substance. Proximate organic analysis is the determination of the compounds present in a mixture, or of the radicals present in a compound.

Both ultimate and proximate analyses are often required in the examination of organic materials. In the case of a complex mixture, however, proximate analysis is frequently directed to the determination of the principal groups of related compounds rather than of each individual compound present.

In the analysis of ordinary animal and vegetable substances it is usually assumed that all of the organic matter is made up of not more than six elements — carbon, hydrogen, nitrogen, oxygen, sulphur and phosphorus. Other elements present would usually be found in the ash, though not always without loss as will be explained later.

While the results of ultimate analysis are often calculated to the basis of dry, ash-free substance, it is not to be supposed that the ash like the moisture is simply mixed with the organic matter of the original sample. In many cases the inorganic compounds found in the ash are formed during combustion, the bases having existed in combination with organic acids or proteids while the acid radicals may also have existed in organic combination or may have been formed by the oxidation of the carbon, sulphur and phosphorus of the organic matter.

The preliminary treatment of samples and the most generally applicable methods of ultimate analysis including the preparation and analysis of ash, will be briefly outlined in this chapter. In the one following, those methods which are more particularly adapted to the determination of nitrogen, sulphur and phosphorus in animal and vegetable substances will be more fully described.

### PRELIMINARY TREATMENT OF SAMPLES.

Samples received and analyzed in liquid form should be kept in well-filled tightly-stoppered bottles and so far as possible in a cool

dark place. If the liquid already contains an antiseptic or is rich in any such preservative substance as salt, glycerol or alcohol, or if it can be kept at a sufficiently low temperature, no further precautions regarding the preservation of the sample will be required.

When there is danger of fermentation the sample should either be sterilized by heat or preserved by freezing or by the addition of an antiseptic, the latter method being usually the most practical. The choice of antiseptic will depend upon the nature of the sample and of the analysis to be made. Mercuric chloride, hydrogen peroxide, formaldehyde, salicylic acid, chloroform and thymol are among the most useful preservatives. Formaldehyde added in the proportion of 0.2 to 0.3 c.c. of the commercial forty per cent. solution to each 100 c.c. of sample is especially convenient and efficient but cannot be used in all cases as it may interfere with some of the tests to be made in the course of the analysis.

No fixed rules can be given for the preliminary treatment of solid samples but the following directions will apply in most cases.

If the sample is moist, dry it thoroughly in a boiling water oven, allow to cool in the air, note the loss in weight and use this "air-dry" sample for analysis. Always determine moisture in the air-dry sample so that the analytical results can be calculated to the "water-free" basis if desired.

An ordinary drug or coffee mill * is usually better than a mortar for grinding the air-dry sample. For proximate analysis of plant or animal tissues the preliminary grinding should theoretically be so thorough as to break all of the cells. In most cases it is found sufficient to grind until the sample passes a sieve having holes one-half millimeter in diameter.

Substances too tough or sticky to be ground by ordinary means can often be prepared by rasping or by grinding in a mortar with a known proportion of sharp sand or broken glass.

## OUTLINE OF ULTIMATE ORGANIC ANALYSIS.

Ultimate organic analysis is discussed in so many well-known laboratory manuals that no attempt will be made to include the subject here. For comparison with the methods to be given in

* For laboratories where many fibrous samples are to be ground the Dreef mill is very advantageous. A description of this and other special forms of grinding apparatus will be found in Wiley's Agricultural Analysis, Volume III, Part First. (Easton, Pa., 1897).

he next chapter, however, the most generally applicable processes vill be mentioned with references to some of the more complete or ecent descriptions.

In the determination of carbon and hydrogen the sample is isually burned in a combustion tube in a current of air or oxygen, he products of combustion being led over hot copper oxide to ensure omplete oxidation. The resulting water and carbon dioxide are ibsorbed respectively, either by calcium chloride and potassium iydroxide or by sulphuric acid and soda-lime. This method equires modification when the sample contains halogens, light netals, or considerable amounts of nitrogen or sulphur. For a liscussion of such modifications as well as for the essential details of the process, the works of Benedict,* Hans Meyer † and Lassar-Cohn ‡ as well as older standard works should be consulted.

For the determination of nitrogen the Dumas method alone is ipplicable to all organic compounds. The substance is burned vith copper oxide and the products of combustion passed over hot netallic copper. The nitrogen gas thus obtained is measured and ts weight calculated. For details of this method see Gattermann,§ Hans Meyer (*l. c.*), or the methods of the Association of Official Agricultural Chemists.‖

For the determination of sulphur and phosphorus, the well-known method of Carius is considered the most generally applicable. The substance is oxidized by heating with fuming nitric icid in a sealed tube and the resulting sulphuric and phosphoric icids are determined by the usual methods. The manipulation of he Carius method is fully described by Gattermann.

Ultimate analyses can also be made by burning the substance vith an excess of oxygen in a closed chamber such as the bomb ised for calorimetric work or a specially constructed small autoclave. This method, proposed by Berthelot ¶ and developed largely by Hempel,** has been used extensively in fuel analyses by Lang-

---

* Elementary Organic Analysis, Easton, Pa., 1900.

† Analyse and Konstitutionsermittlung organischer Verbindungen, Berlin, 1903.

‡ Arbeitsmethoden für Organisch-chemische Laboratorien [3 Aufl.], Hamburg, 903.

§ Practical Methods of Organic Chemistry, New York, 1901.

‖ Bulletin 46, Bureau of Chemistry, U. S. Department of Agriculture.

¶ *Compt. rend.*, 1892, 114, 318; 1899, 129, 1002; *Ztschr. anal. Chem.*, 1901, 40, 124.

** *Ber. deut. chem. Ges.*, 1897, 30, 202.

bein.* Its application to the determination of sulphur is given in the next chapter.

## PREPARATION AND ANALYSIS OF ASH.

### PREPARATION OF ASH.

Incineration of ordinary substances is best accomplished in a flat platinum dish in a muffle. The substance should first be carefully charred at the front of the muffle, then burned until white or very light gray at a temperature of 500° to 600°.† If the temperature cannot be accurately known, the operation should be carefully watched and the dish never heated above a very low redness. Higher temperatures not only increase the loss of chlorides but may cause the ash to fuse over some of the carbon and thus greatly retard its combustion. If the substance is rich in salts of the alkalies, char it gently, extract with warm water, pour off the aqueous extract and set aside while burning the carbonaceous residue to whiteness, then return the aqueous extract to the platinum dish, evaporate to dryness, and ignite gently as already directed. Animal substances, seeds, and other samples rich in phosphates should be extracted with acetic acid instead of with water alone.

Various forms of special apparatus for the preparation of ash have been described, those of Shuttleworth,‡ Tucker§ and Wislicenus || being probably the best known.

In order to avoid discrepancies due to the absorption of moisture from the air, the ash may be thoroughly mixed while still warm and transferred at once to a tightly stoppered weighing bottle. The ash being then perfectly dry, no determination of moisture is necessary and the portions required for analysis may be weighed out by difference.

### METHODS OF ASH ANALYSIS.

Since almost any of the elements found in soil or water may alsc be found in ash, no attempt to outline a complete scheme o

* *Ztschr. angew. Chem.*, 1900, 1227, 1259; 1901, 516.

† In a series of experiments by Thompson and the writer in which portions of drie milk were incinerated at known temperatures in an electrically heated muffle, it wa found that increase of temperature from 450° to 600° had no appreciable influence upo the amount of chlorine recovered in the ash, but that a loss occurred at about 650°. Fc other results of this study see the chapter on milk analysis.

‡ Dissertation, Goettingen, *1899*. *Journ. Landw.*, 1899, 47, 173.

§ *Ber. deut. chem. Ges.*, 1899, 32, 2583.

|| *Ztschr. anal. Chem.*, 1901, 40, 441.

analysis would be of much practical use, especially since the amount of ash available is generally so small that the analysis must be limited to the determination of the more important constituents. The determinations most often required may be carried out in accordance with the plan adopted by the Association of Official Agricultural Chemists (*l. c.*). Methods of ash analysis are also described in Fresenius' *Quantitative Analysis* and in a paper by Tollens in the *Experiment Station Record*, Volume XIII, pages 207–220. For detailed descriptions of the separations and determinations required, text-books on inorganic quantitative analysis * should be consulted. Small amounts of iron may conveniently be estimated by colorimetric methods.†

## Interpretation of Ash Analysis.

*Chlorine.* — Even when the incineration has been carried on carefully at temperatures considerably below those at which the chlorides begin to volatilize, the whole of the chlorine of the original substance is rarely recovered in the ash.‡ Apparently this loss of chlorine is due to the formation of acid products in the early stages of ignition of the organic matter, when the conditions approach those of dry distillation. When the relative amount of organic matter is large these acid products are formed in such quantity as to expel a considerable part of the chlorine from its combination with the alkalies. The alkali thus retained in combination with organic matter is converted to carbonate by further ignition, and this in turn may be acted upon by some stronger acid formed during the combustion. The loss of chlorine increases with the proportion of organic matter to chloride. According to Davis § it may be prevented by the addition of sodium carbonate to the extent of 5 per cent. of the weight of organic matter present, but this of course is not permissible in the preparation of ash for general

* Among the more recent are — Classen: Ausgewählte Methoden der Analytischen Chemie, Braunschweig, 1901–1903. Treadwell: Analytical Chemistry, Vol. II (Quantitative Analysis), New York, 1904. Miller: Quantitative Analysis for Mining Engineers, New York, 1904.

† Thompson: *Journ. Chem. Soc.*, 1885, 47, 493. Bornträger: *Chem. Ztg.*, 1896, 20, 398. Richards and Woodman: Air, Water and Food, New York, 1904. Pulsifer: *Journ. Amer. Chem. Soc.*, 1904, 26, 967.

‡ For a very full discussion of the loss of chlorine in the preparation of ash, including references to earlier work see Adlerskron: *Ztschr. anal. Chem.*, 1873, 12, 390.

§ *Journ. Soc. Chem. Ind.*, 1901, 20, 98.

analysis. Shuttleworth * recommends calcium acetate as being efficient in preventing loss of chlorine and especially convenient in the incineration of siliceous substances such as straw.

*Carbonates.* — Carbonates in ash are not derived necessarily from salts of carbonic or organic acids existing in the original substance. The bases found as carbonates in the ash may have been combined with proteids or may have been derived from inorganic salts decomposed by heating in contact with the large excess of organic matter, as just mentioned in the case of alkaline chlorides. On the other hand, carbonates originally present or formed during ignition may be changed to sulphates or phosphates by the action of acids resulting from the oxidation of organic sulphur or phosphorus.

*Sulphates.* — The amount of sulphate found in the ash bears little relation either to the total sulphur, or to that existing as sulphate, in the original substance. Depending upon the other constituents present and the conditions of incineration, sulphates may be reduced or converted to phosphates, or, on the other hand, the organic sulphur may be largely oxidized and retained as sulphate. Very rarely in the combustion of natural products does all of the sulphur remain in the ash. More frequently only a small fraction of it is thus retained.

*Phosphates.* — The phosphates of the ash usually represent the whole of the pre-existing inorganic phosphates and, in the case of natural substances, usually much of the phosphorus which was present in organic combination. Since the latter exists mainly, if not entirely, as organic phosphates or substituted phosphoric acid, it is largely burned to free orthophosphoric acid and thus remains as mineral phosphate in the ash. With many substances, practically all of the phosphorus is thus retained as phosphate by the ash, but it cannot be assumed that such will be the case whenever there are bases enough to combine with all of the phosphoric acid. Thus there is some loss of phosphorus in burning the dried residue of milk at 600°, although the ash thus obtained contains a considerable proportion of chlorides. When the phosphoric acid formed is more than sufficient to saturate the bases present, the excess of acid is very slowly volatilized. In such cases the ash is apt to fuse over the last portions of unburned carbon, interfering both with the determination or preparation of ash and with the determination of carbon in the elementary analysis.

* Dissertation, Goettingen, 1899.

*Bases.* — Some of the heavy metals, if present, may be reduced and volatilized during the combustion of the organic matter. There is danger of mechanical loss of ash if combustion is carried on rapidly or in too strong a current of air. A sufficient circulation of air will be secured by inclining slightly the muffle in which the substance is burned. The volatilization of alkaline chlorides as such at temperatures of 650° or over has already been mentioned.

When small amounts of metallic bases are to be determined it is usually advantageous and often necessary to destroy the organic matter, in part at least, by boiling with acids instead of by burning in the air.*

* Neumann: *Ztschr. physiol. Chem.*, 1902–3, 37, 115. Meilliere: *Journ. Pharm. Chim.*, 1902, [6] 15, 97. Munson: U. S. Dept. Agr., Bur. Chem., Bull. 65, p. 53.

## CHAPTER II.

## Nitrogen, Sulphur and Phosphorus.

### THE DETERMINATION OF NITROGEN.

The method of Dumas as outlined in the preceding chapter has the advantage of being applicable to all classes of nitrogen compounds. In the great majority of cases, however, it is equally accurate and much more convenient to use one of the methods based upon the conversion of nitrogen to ammonia and the determination of the latter by titration.

Before the introduction of the Kjeldahl process, the soda-lime method of Will and Varrentrap was commonly used. In this method the finely ground substance is mixed with a large excess of soda-lime and heated in a combustion tube, the ammonia given off being absorbed in standard acid. In order to ensure the reduction of nitro-compounds or nitrates, this method was modified by the introduction of stannous sulphide (Goldberg); of sodium formate and sodium thiosulphate (Arnold); or of sodium thiosulphate and a mixture of equal parts sulphur and powdered sugar or charcoal (Ruffle). The soda-lime method has now been very generally superseded by the various modifications of the Kjeldahl process.

### THE KJELDAHL METHOD.

In this process the substance is decomposed by heating with strong sulphuric acid, usually with the addition of some reagent which assists the decomposition either by raising the boiling point or by acting as a carrier of oxygen. When decomposition is complete the nitrogen remains as ammonium sulphate in the sulphuric acid, the carbon and hydrogen of the substance having been oxidized and the products of oxidation boiled out of the solution. The oxidation takes place partly at the expense of the sulphuric acid so that a considerable evolution of sulphur dioxide occurs, especially in the earlier stages of the process. After the completion of the digestion, the ammonia is liberated by means of fixed alkali and determined by distilling into standard acid. In the various modifications of the process, different reagents or combinations of reagents are used to hasten the decomposition of the organic matter by the sulphuric acid.

Kjeldahl originally directed * that the substance be heated with sulphuric acid, with or without the addition of phosphoric anhydride, until a clear solution is obtained; then potassium permanganate added in small portions to the hot solution until it remains permanently colored, the permanganate being added very cautiously on account of the danger of a loss of nitrogen if the reaction becomes too vigorous.

Willfarth † introduced the use of mercuric oxide to facilitate the action of the sulphuric acid, and stated that if the solution be boiled until colorless neither phosphoric anhydride nor potassium permanganate need be used. In his earlier experiments Willfarth used copper instead of mercury. Arnold ‡ used both mercury and copper in addition to phosphoric anhydride.

Gunning § used for the digestion a simple mixture of sulphuric acid with one-third to one-half its weight of potassium sulphate. Arnold and Wedemeyer ‖ modified the Gunning process by the use of mercury and copper in addition to the potassium sulphate. By this modification the time required to decompose the organic matter was greatly reduced and good results were obtained with a number of alkaloids and other compounds which had not readily yielded the whole of their nitrogen when treated by the methods previously used. Independently Dyer ¶ obtained equally good results on a wide range of organic compounds by the use of mercury and potassium sulphate without copper.

The official agricultural chemists authorize ** the Kjeldahl-Willfarth and the Gunning methods for the analysis of foods, spices other than peppers, and fertilizers not containing nitrates. For peppers, to secure complete ammonification of the alkaloidal nitrogen, the Arnold-Wedemeyer modifidation of the Gunning method was provisonally adopted in 1902. In the experience of this laboratory, the Dyer modification has been found more rapid and slightly more accurate than either the Kjeldahl-Willfarth or Gunning method and fully as efficient in the case of alkaloids as is the Arnold-Wedemeyer method, while it has the advantage

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* *Ztschr. anal. Chem.*, 1883, **22**, 366.

† *Chem. Centrbl.* 1885 [3] **16**, 17, 113.

‡ *Archiv. der Pharm.* [3], **24**, 785; *Ztschr. anal. Chem.*, 1887, **26**, 249.

§ *Ztschr. anal. Chem.*, 1889, **28**, 188.

‖ *Ztschr. anal. Chem.*, 1892, **31**, 525.

¶ *Journ. Chem. Soc.*, 1895, **67**, 811.

** Bulletins 46 and 65, Bureau of Chemistry, U. S. Dept. Agriculture.

over the latter of requiring one less reagent and of yielding a colorless solution.

*Applicability of the Kjeldahl Method.*—The Gunning-Arnold-Dyer modification, which for the reasons just mentioned is recommended for general use, is applicable to all classes of animal and vegetable substances, including such difficultly decomposable bases as betaine and the pyridine and chinoline alkaloids, and to cyanides, ferrocyanides and ferricyanides. It has also been tested * with good results on many other compounds, including acetanilid, sulphanilic acid, orthobenzoic sulphinid, amidobenzoic acid, benzamid, diamidophenol, naphthylamine, diphenylamine, diphenylthiourea, nitroso-dimethylaniline, indigotin, pyridine, and oxyphenyl methylpyrimidine.

Jodlbauer's modification,† devised for the determination of nitrogen in nitrates, was found by Dyer to be applicable to nitro-compounds, to azo-, hydrazo- and amidoazo-benzene, to carbazol and, with the addition of 1 or 2 grams of sugar, to hydroxylamine and oximes. Dyer did not obtain the whole of the nitrogen of hydrazine derivatives but Dafert ‡ and Milbauer § have published modifications which are said to give accurate results with this class of compounds.

### GUNNING-ARNOLD-DYER MODIFICATION.

*Reagents.*— Pure concentrated sulphuric acid. Mercury. Pure potassium sulphate. Potassium sulphide solution, 40 grams per liter. Saturated solution of sodium hydroxide (commercial). Granulated zinc or pumice stone. Paraffin. Solution of methyl-orange, congo red, cochineal, lacmoid, or any other indicator suitable for titration in the presence of ammonium salts. Standard solutions of acid and alkali, preferably one-fifth or one-tenth normal.

*Determination.* — Weigh 0.5 to 5.0 grams sample, or so much as will probably yield from 50 to 75 milligrams of ammonia, and transfer to a pearshaped Kjeldahl flask of 550 to 750 c.c. capacity. Add 20 to 25 c.c. concentrated sulphuric acid and about 0.7 gram of mercury. Place the flask in an inclined position and heat gently until the first vigorous frothing ceases, then raise the heat grad-

* In some cases without the use of mercury. See in addition to the papers already cited, that of Gibson in the *Journal of the American Chemical Society*, 1904, 26, 105.

† *Chem. Centrbl.*, 1886, 3, 17, 433.

‡ *Landw. Versuchs-Sta.*, 1887, 34, 311.

§ *Ztschr. anal. Chem.*, 1903, 42, 725.

ually until the liquid boils; remove the flame for a few minutes, add 10 to 12 grams of potassium sulphate and boil. If the liquid is kept actually boiling, it will usually be clear and colorless within 30 minutes after the addition of the sulphate. Continue the boiling for at least 30 minutes after the solution becomes colorless or in any case for one hour from the time the potassium sulphate is added. If the sample contains alkaloids, the boiling should be continued for at least three hours in all and not less than two hours after the solution is colorless. When the digestion is finished, allow the flask to cool for 10 to 15 minutes or to 40°–60° (if allowed to become thoroughly cold the solution solidifies); then dilute carefully with 150 to 200 c.c. of water; allow to cool, add 25 c.c. potassium sulphide solution, mix well and then add 75 to 100 c.c. (or enough to make the reaction strongly alkaline*) of a cold saturated solution of sodium hydroxide, pouring it carefully down the side of the flask, so that it does not mix immediately with the acid solution. Add a few pieces of granulated zinc or pumice stone to prevent bumping, and a piece of paraffin the size of a pea to diminish frothing; connect the flask (preferably by means of a Hopkins' distilling head) with a condenser, the delivery tube of which dips into 50 c.c. of N/10 sulphuric or hydrochloric acid or its equivalent in the receiver; mix the contents of the flask by shaking and distil until about one-half of the liquid has passed into the receiver. Titrate the excess of acid in the receiver by means of standard alkali, using one of the indicators mentioned above.

The reagents used should be tested by making a blank determination with pure sugar or cellulose, carrying out all operations in exactly the same way as in a regular analysis.

*Notes.*—If in transferring the substance to the flask any particles or drops should lodge in the neck, they can be washed down by the sulphuric acid subsequently added. Dry samples can usually be weighed on a watchglass and brushed into the flask through a funnel having a wide stem which has been cut down to a length of about one cm., or transferred by means of a narrow strip of glazed paper. It is often more convenient to weigh the sample on, or transfer to, a small piece of pure filter paper, fold the latter loosely over the weighed portion and introduce the whole into the flask in such a way that the sample can be easily shaken

* Corallin (rosolic acid) may be used as indicator to show that the contents of the flask are alkaline before beginning the distillation.

free from the paper. The amount of cellulose thus introduced with the sample does not materially prolong the time required for digestion, while its reducing effect may aid in the ammonification of any firmly bound nitrogen present. Time should be allowed for thorough wetting of the sample by the sulphuric acid before heat is applied. During digestion a small funnel or balloon stopper may be placed loosely in the mouth of the flask to retard the evaporation of acid and guard against mechanical loss. The flask may rest upon a wire gauze, an iron plate or a piece of asbestos having a circular hole about 4 cm. in diameter which permits the free flame of the Bunsen burner to play upon the flask below the surface of the boiling liquid. The digestion should be conducted in a well ventilated hood where the air is free from any considerable amount of ammonia. In order to hasten the decomposition of organic matter, Dakin * and Milbauer (*l. c.*) recommend the use of potassium persulphate, while Krüger † suggests the addition of potassium dichromate in excess of the amount necessary to oxidize all carbon and hydrogen present. The method here described is, however, sufficiently rapid and more convenient for ordinary work.

The precipitation of mercury as sulphide before rendering the solution alkaline is to prevent the formation of mercur-ammonium compounds which do not readily yield their ammonia on boiling with caustic alkali. An ordinary Liebig condenser can be used in this distillation, though block tin tubing is generally preferred as being more resistant to the action of steam and ammonia. Unless the condenser tube is straight and nearly vertical, it is safer to rinse it out with a little cold distilled water at the end of the distillation and add the rinsings to the distillate. The distillation as usually carried out requires from forty minutes to one hour. Only about one third of this time is actually required to expel the ammonia from the boiling solution and if it is desired to hasten the operation, the steam can be conducted through tin tubing without condensation into a cooled receiver; or, as suggested by Benedict,‡ the water which cools the condenser may be drawn off after the solution has been boiling 15 minutes, and the boiling continued until enough steam has passed through to carry all of the ammonia from the condenser tube into the receiver. Fixed alkali is prefer-

* *Journ. Soc. Chem. Ind.*, 1902, 21, 848.

† *Ber. deut. chem. Ges.*, 1894, 27, 609.

‡ *Journ. Amer. Chem. Soc.*, 1900, 22, 259.

able to ammonia for the final titration since the latter reagent is liable to lose strength during use, probably through evaporation of ammonia from the tip of the burette and from the falling drop.

The reasons for preferring the method as above described to the original Kjeldahl-Willfarth or Gunning method have been briefly outlined above. If either of the latter methods is used, the same details of manipulation may be followed except that the digestion should be continued for at least two hours after the solution becomes colorless and not less than three hours in all. Even with this longer time of boiling the results are often slightly low. For the results of a comparative study of the Kjeldahl-Willfarth, the Gunning, and the above described method with special reference to the time of boiling required see the *Journal of the American Chemical Society* for April and November, 1904.

### Method for Nitrates and Nitro-compounds.

The loss of nitric acid which might otherwise occur when sulphuric acid is poured upon a sample containing nitrates, is avoided by having present some substance which is very easily nitrated, such as salicylic acid, benzoic acid or phenol. The nitrogen having been thus fixed as a nitro-compound, the latter may be reduced by the addition of zinc dust (Jodlbauer) or sodium thiosulphate (Forster) after which the determination is carried out in the usual manner.

*Reagents.* — Sulphuric-salicylic acid, prepared in advance by dissolving salicylic acid in pure concentrated sulphuric in the proportion of 2 grams of the former to 30 c.c. of the latter. Zinc dust, an impalpable powder; or crystallized sodium thiosulphate. Other reagents as for the method above described.

*Determination.*—To the weighed portion of substance in a Kjeldahl flask, add 30 c.c. of the sulphuric-salicylic acid solution and shake until thoroughly mixed; then add 5 grams crystallized sodium thiosulphate, or add gradually with constant shaking 2 grams of zinc dust; warm gently until frothing subsides and then boil until thick white fumes cease to be given off. Allow to cool somewhat, add mercury and potassium sulphate and complete the process as described above. Or the potassium sulphate may be omitted and the oxidation completed by means of permanganate as in the 'modified Kjeldahl' method of the official agricultural chemists.

*Notes.*—Thiosulphate is the more convenient reducing agent

and can be safely used if the amount of nitric nitrogen is small. Zinc dust appears to be a safer reagent for samples rich in nitrates. In the presence of considerable amounts of chloride or of ammomium salts there is danger of a loss of nitrogen as oxide when the sample is treated with the sulphuric-salicylic acid mixture. In such cases the latter mixture should be thoroughly cooled before using and then poured as quickly as possible over the sample and the flask allowed to stand with occasional shaking for at least half an hour before the reducing agent is added. Ten minutes should be allowed for the reduction to take place before the solution is heated. With these precautions the loss of nitrogen will be reduced to an amount too small to be appreciable in ordinary work.

## THE DETERMINATION OF SULPHUR.

In the various methods employed for the determination of sulphur, organic matter is destroyed by oxidation and the resulting sulphuric acid is precipitated and weighed as barium sulphate in the usual way. The chief difficulty lies in securing complete oxidation without loss, since many substances contain sulphur in forms from which it is easily liberated as volatile compound.

### COMPARATIVE OUTLINE OF METHODS.

The principal methods of bringing about the oxidation of the organic matter are:

1. By heating with nitric acid or an oxidizing acid mixture.

2. By burning in a combustion tube in a current of air, oxygen or some oxidizing vapor.

3. By heating in the presence of alkali and completing the reaction by the addition of an oxidizing agent.

4. By combustion in oxygen in a closed vessel.

Since the choice of a method depends on the nature of the sample to be examined and the apparatus at hand, no fixed rules can be laid down for this purpose. Several of the more important methods are therefore outlined and three methods which are especially useful for plant and animal substances are given in full.

Of methods of the first group, that of Carius, which has already been mentioned in the previous chapter, is the most important. It is applicable to volatile as well as to non-volatile substances, but is somewhat troublesome and tedious, and determinations are frequently lost through breaking of the heated tubes, especially when

much gas is produced by the oxidation of the carbon of the sample. For this reason only a small amount of any highly carbonaceous substance can be taken for analysis and often the amount of sulphuric acid obtained is too small for satisfactory determination.

Several methods belonging to the second group have been proposed. Among the more important are: (*a*) The method of Brugelmann,* in which the substance is burned in a current of oxygen, complete oxidation being insured by passing the mixture of gases over hot platinum, and absorbing the sulphuric acid in a column of soda-lime. If the substance is poor in sulphur several portions can be burned before dissolving out the lime and determining the sulphuric acid. (*b*) Mixter's modification† of Sauer's method. This consists in burning the substance in a current of oxygen and passing the products of combustion into bromine water. (*c*) Clässon's ‡ method of burning in a current of nitric oxide. (*d*) Barlow's method § in which the substance is burned in a current of oxygen in a specially constructed tube and complete oxidation is insured by means of a second current of oxygen introduced laterally at a point between the boat and the absorbing column. Methods of this type, carefully carried out, ensure complete oxidation and several portions of a sample may be burned in succession so as to secure a larger amount of sulphuric acid for determination. They are, however, less rapid than the methods described below, which are usually quite as accurate.

Liebig destroyed organic matter and converted sulphur to sulphate by heating with a mixture of caustic alkali and alkaline nitrate and methods of this type have been very generally used for plant and animal materials and other non-volatile substances. Of the many modifications of Liebig's method, the use of sodium peroxide as an oxidizing agent is most important. The peroxide may be used in connection with hydroxide as in Osborne's method, or the sample may be mixed at once with an excess of peroxide and ignited in a closed crucible ‖ or in the combustion chamber of the Parr calorimeter.¶

* *Ztschr. anal. Chem.*, 1876, **15**, 1, 175; 1877, **16**, 1.

† *Amer. Chem. Journ.*, 1880, **2**, 396.

‡ *Ber. deut. chem. Ges.*, 1886, **19**, 1910; 1887, **20**, 3065.

§ *Journ. Amer. Chem. Soc.*, 1904, **26**, 341.

‖ Sundstrom: *Journ. Amer. Chem. Soc.*, 1903, **25**, 184. Pennock and Morton: *Ibid.*, **25**, 1265.

¶ Parr: *Journ. Amer. Chem. Soc.*, 1900, **22**, 646; 1904, **26**, 1139. Konek: *Ztschr. angew. Chem.*, 1903, 516. Leclerc and Dubois: *Journ. Amer. Chem. Soc.*, 1904, **26**, 1108.

Berthelot, in proposing the use of the oxygen calorimeter for the elementary analysis of organic materials,* stated that on combustion with 25 atmospheres of oxygen the sulphur was completely oxidized and in the presence of moisture could be quantitatively recovered as sulphuric acid. This method has been found both convenient and accurate. Complete oxidation takes place instantaneously, and since the combustion chamber is hermetically sealed there is no possibility of a loss of volatile compounds. As noted in the preceding chapter, a small autoclave may be used for the combustion if a bomb calorimeter is not available. According to Hempel,† the sample can be burned in a large glass bottle filled with oxygen at atmospheric pressure.

The nature and number of the samples to be analyzed will usually determine which of the above methods is to be preferred. For the majority of cases either Liebig's or Osborne's method, or if the facilities are available, Berthelot's method of combustion in compressed oxygen may be recommended.

### Liebig's Alkali Method.

In a large silver or nickel crucible mix about 8 grams potassium hydroxide and about 1 gram potassium nitrate. Fuse and stir with a silver or nickel spatula. Allow to cool and add 1 to 2 grams of the finely pulverized sample. Heat gently and as soon as the mass softens, stir well so as to bring the whole of the sample into contact with the alkali before it is strongly heated, then gradually increase the heat, stirring frequently to keep down frothing, and continue heating until the mass becomes colorless. For samples which react violently when stirred into the alkali as above, a smaller proportion of nitrate should be used at the beginning, or the nitrate may be omitted at the start and added after the sample has been mixed with the fused hydroxide. If the substance is not quickly oxidized by the fusion mixture, small amounts of pulverized nitrate may be added from time to time and the heating continued with frequent stirring. The fusion will usually be perfectly colorless when the oxidation of organic matter is complete. After cooling, dissolve the fusion in water in a beaker or casserole, acidulate with hydrochloric acid and evaporate to dryness to expel nitric

* *Compt. Rend.*, 1892, 114, 318; 1899, 129, 1002. See also Hempel: *Ber. deut. chem. Ges.*, 1897, 30, 202, and Langbein: *Ztschr. angew. Chem.*, 1900, 1227, 1259.

† *Ztschr. angew. Chem.*, 1892, 393. See also Graeffe: *Ibid.*, 1904, 616.

and nitrous acids and heat at 110° to dehydrate silica if present. If much nitrate or silica was present the evaporation with hydrochloric acid should be repeated. Dissolve the residue in cold water, adding a few drops of hydrochloric acid, filter if not perfectly clear, heat the solution to boiling and precipitate at the boiling temperature by adding a solution of barium chloride, drop by drop with constant stirring, until in considerable excess. Boil 5 or 10 minutes longer and allow to stand in a hot place until the precipitate settles, leaving the supernatant liquid perfectly clear. Filter and wash the barium sulphate, first with water acidulated with hydrochloric acid, then with water till free from chlorides and finally ignite and weigh with the usual precautions.

*Notes and Precautions.* — The reagents should be tested by making a "blank" determination with sugar or cellulose. Do not reject this blank if no precipitate appears when the barium chloride is added, but allow the solution to stand over night to ensure the separation of any small amount of barium sulphate present, then filter, wash, ignite and weigh, and subtract the weight from that of the precipitate found in the determination. An apparently trifling amount of precipitate in the "blank" may weigh enough to appreciably affect the results. If the fusion is heated by an ordinary gas flame it is likely to absorb sulphur compounds from the latter. To avoid this use an alcohol flame for heating the fusions. It cannot be expected that the blank determination should show accurately the sulphur absorbed from the gas flame. Even when an alcohol flame is used and the sulphur obtained from the reagents is deducted, the results are sometimes too high. According to Keiser * this is due chiefly to the fact that the fused alkali takes up some silver from the crucible and, on acidifying with hydrochloric acid, the silver chloride formed is held in solution by the excess of potassium chloride present and is afterward carried down by the barium sulphate, increasing the weight of the precipitate. Keiser therefore recommends that the neutralized solution be cooled, diluted with water to about a liter and allowed to stand. Any silver chloride present will then precipitate and may be filtered out before adding barium chloride.

A modification which is often useful consists in heating the substance with strong nitric acid until a considerable part of the organic matter has been oxidized, after which an excess of alkali is

* *Amer. Chem. Journ.*, 1883, 5, 207.

added and the mixture transferred to a crucible and carefully fused until oxidation is complete. Hammarsten* found this method to give the same results as the methods of Liebig and of Clässon when applied to casein, egg albumen and gelatin.

### Osborne's Peroxide Method.†

Convert about 10 grams of sodium peroxide into hydroxide by adding to it, in a silver or nickel crucible, a slight excess of water and heating until the excess is boiled off. Allow the fusion to cool until pasty, then add one to two grams of the sample and stir it into the alkali as quickly as possible. Heat gently and stir well to keep down the frothing. When the first vigorous action is over, heat until the mass fuses and stir in small portions of fresh peroxide until decomposition is complete and the fusion is practically colorless. Then allow to cool, dissolve the fusion and acidulate with hydrochloric acid; boil to destroy any excess of peroxide and to expel chlorine and complete the determination as in Liebig's method.

*Notes.* — Blank determinations must be made as in the case of the Liebig method, and the same precautions observed to prevent the absorption of sulphur compounds from gas flames or from the air of the laboratory. There is also the same danger of high results from contamination of the barium sulphate precipitate. If silica is absent the acidulated solution need not be evaporated to dryness since no nitrate is used in the fusion. For all such cases the method is considerably more rapid than that of Liebig, though requiring somewhat more careful attention during the fusion of the substance with the alkaline oxidizing mixture. This method has been extensively used during recent years in the analysis of proteids, food materials and physiological products and has lately been adopted by the Association of Official Agricultural Chemists. Several methods involving the use of sodium peroxide had previously been proposed, of which that of Asboth ‡ is perhaps the best known. This consists in mixing the substance with 10 grams of dry sodium carbonate and 5 grams of peroxide in a nickel crucible and heating carefully at first and then more strongly until oxidation is complete. After dissolving the fusion in water the

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* *Ztschr. physiol. Chem.*, 1885, **9**, 273.

† *Journ. Amer. Chem. Soc.*, 1902, **24**, 142.

‡ *Chem. Ztg.*, 1895, **19**, 2040.

solution may be boiled with bromine to ensure complete oxidation. The alkaline mixture here used being much less fusible than sodium or potassium hydroxide, the method should be used only for such substances as do not readily lose sulphur on heating. For such substances it offers the advantage of simplicity in manipulation.

### BERTHELOT'S OXYGEN METHOD.

Press into the form of a pellet a suitable amount of the substance (usually one to three grams), introduce into the bomb calorimeter and burn in oxygen under a pressure of about 25 atmospheres in the same manner as in the determination of heat of combustion.* Screw into the exit tube of the bomb a coupling carrying a delivery tube of about 0.5 mm. internal diameter and connect with a washing cylinder containing a little water. Carefully open the valve and allow the gas to bubble through the water until the contents of the bomb reach atmospheric pressure; then disconnect, open the bomb and rinse out all moisture which has condensed in the chamber, on the lining of the cover, or on the rods which support the combustion capsule. This must be done carefully as the rinsings usually contain the greater part of the sulphur. In order to keep down the volume of the solution, the water from the washing cylinder may be used for the first rinsing. Dissolve any ash found in the combustion capsule in a little hydrochloric acid, remove silica, if necessary, and add the solution to that obtained by rinsing the bomb. Examine the lead gasket in the cover of the bomb and if a slight film of sulphate is found, wash it into the main solution.† Finally boil this solution down to the desired volume, neutralize any excessive amount of hydrochloric acid which may have been introduced with the solution of the ash, filter if necessary and determine the sulphuric acid by precipitation with barium chloride as already described.

*Notes.*— This method, based mainly upon the suggestion of Berthelot and the subsequent work of Hempel, has been applied ‡ with very satisfactory results to a number of animal and vegetable substances as well as to synthetic organic compounds containing sul-

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* Atwater and Snell: *Jour. Amer. Chem. Soc.*, 1903, 25, 659.

† Such minute quantities of lead sulphate as are ordinarily found will dissolve readily in the solution and will not interfere with the precipitation of sulphuric acid as barium sulphate.

‡ *Jour. Amer. Chem. Soc.*, 1902, 24, 1100.

phur in forms not readily oxidized to sulphuric acid — *e. g.*, benzyl sulphide and diphenyl-thiourea. So far as tested, no cases of incomplete combustion have been found. If this were suspected, the gas drawn off after the combustion should be led through bromine water or an alkaline bromine solution and the washings of the bomb added to this solution and boiled to insure oxidation of sulphites to sulphates. Unless the bomb is emptied gradually in some such way as has been described, there is danger that some sulphuric acid may be carried away mechanically by the escaping gas. This is probably the principal cause of the low results occasionally reported. When properly carried out this method has several important advantages. Combustion takes place instantaneously and with no chance for the escape of volatile products. The products of combustion can be drawn off and examined as desired. The only reagents required in obtaining the sulphur as sulphuric acid are compressed oxygen and hydrochloric acid, both of which are easily obtained free from any appreciable amounts of sulphur. By eliminating the use of alkali, any danger of absorption of sulphur compounds from the air is avoided, and no considerable amount of foreign salts is introduced into the solution from which the sulphuric acid is to be precipitated.

## THE DETERMINATION OF PHOSPHORUS.

The oxidation of organic matter for the determination of phosphorus may be accomplished by any of the methods described in connection with the sulphur determination. It is of course unnecessary in this case to avoid the use of gas flames and with most substances there is much less danger of loss by volatilization than in the case of sulphur. For these reasons and because of the care required in dissolving the fused residue of phosphates left in the ignition capsule, the compressed oxygen method has no such marked advantages in the determination of phosphorus as in that of sulphur.

### Alkali Methods.

Liebig's method of oxidation, either with or without previous digestion with nitric acid, or oxidation by peroxide may be used but the use of sodium carbonate, as in the following method, is generally more convenient.

Mix 1 to 3 grams substance with 6 to 7 grams dry sodium carbonate in a platinum dish and spread 3 or 4 grams of the carbonate

over the mixture. Heat over a Bunsen burner, carefully at first until frothing ceases, then strongly until the mass fuses. To the fusion add small portions of pulverized potassium nitrate, stirring thoroughly with a platinum rod or spatula, until the mass is entirely colorless. The whole amount of nitrate required does not usually exceed one gram. After cooling, transfer the fusion to a beaker, dissolve in water, add an excess of nitric acid and boil. Allow the solution to cool, neutralize with ammonia, add a few drops of nitric acid and then a moderate excess of molybdate solution.* Digest at about 65° for an hour with occasional shaking or stirring, filter, and wash with cold water or a cold solution of ammonium nitrate. Test the filtrate by adding more molybdate solution and digesting again at about 65°. Place the beaker used for the precipitation under the funnel, dissolve the precipitate through the paper by means of ammonia and wash thoroughly with water, keeping the volume of the solution below 100 c.c. Nearly neutralize with hydrochloric acid, cool thoroughly and add a moderate excess of magnesia mixture,† drop by drop, with constant stirring. After 15 minutes add 10 c.c. of concentrated ammonia or its equivalent; let stand four to twenty-four hours; filter, (preferably on an asbestos felt in a Gooch crucible) wash with dilute ammonia (2.5 to 5 per cent.) until practically free from chlorides, ignite and weigh as magnesium pyrophosphate.

### Neumann's Acid Method.‡

*To 1 to 3 grams substance in a Kjeldahl flask add 20 c.c. con*centrated sulphuric acid and 10 grams of ammonium nitrate and heat carefully until frothing ceases; or heat the sample first with 10 c.c. fuming nitric acid, then cool and add 10 c.c. of sulphuric acid. In either case the solution is boiled with successive additions of ammonium nitrate until organic matter is entirely destroyed and the solution is colorless. If necessary, as many grams of ammonium nitrate may be added as the number of c.c. of sulphuric acid used. Cool the colorless solution, wash it out into a

* Of the usual molybdate solution containing about 5 per cent. of molybdic acid, add at least 50 c.c. for every decigram of phosphoric acid expected.

† If the magnesia mixture is of the usual strength, containing about 5 per cent. of magnesium chloride, add about 15 c.c. for each decigram of phosphoric acid expected.

‡ *DuBois Reymond's Archiv.* (physiol. Abth.), 1897, 552; 1900, 159; Abs. *Chem. Centrbl.*, 1900, I., 571. See also *Ztschr. physiol. Chem.*, 1900, 29, 146; 1902–3, 37 115; *Journ. Amer. Chem. Soc.*, 1902, 24, 1100.

beaker, neutralize with ammonia, add 15 to 20 grams of ammonium nitrate and determine phosphoric acid as in the preceding method, adding a somewhat greater excess of the molybdate reagent.

*Notes.* — This method has been found to give good results with a variety of foods and physiological products. The large amount of sulphates retards the formation of the phosphomolybdate precipitate, but by using liberal quantities of ammonium nitrate and molybdate reagent and allowing at least an hour for the precipitation, the whole of the phosphoric acid is obtained without difficulty. When the substance is first treated with fuming nitric acid, a smaller quantity of sulphuric acid may be used and the phosphomolybdate will then form somewhat more quickly.

In applying this method to liquids such as milk or urine, a suitable amount of sample (25 c.c. milk or 50 c.c. urine,) is transferred to the flask, 20 c.c. sulphuric acid added, and the mixture heated carefully as in the determination of nitrogen until the sample is well charred and the greater part of the water has been boiled off. Ammonium nitrate is then added and the determination is completed as above described.

For additional notes on this method and a discussion of methods for the separation of organic and inorganic phosphorus in plant and animal products, see Hart and Andrews, *Amer. Chem. Journ.* 1903, 30, 470.

## CHAPTER III.

### Alcohols.

Alcohols being usually defined as neutral hydroxyl derivatives capable of reacting with acids to form esters, it follows that the most characteristic alcohol reactions are those involving the replacement of the hydroxyl by an acid radical. For detailed discussions of the analytical behavior of the hydroxyl radical and characteristic reactions of the different groups of alcohols, the reader is referred to the works of Hans Meyer,* while Mulliken's tables † may be followed in the systematic identification of individual pure preparations.

Of the analytical methods involving reactions of the hydroxyl group, the more important are those in which the acetyl or benzoyl ester is formed. The preparation of the dinitrobenzoate serves for the identification of ethyl alcohol as described below. Quantitative methods based on acetylation will be described in the sections on glycerol and the fatty oils. Often, however, esterification is either not quantitative or is less convenient or less delicate than other methods of determination. Thus all monatomic alcohols containing less than four carbon atoms mix freely with water and are not readily separated from it but may be determined in aqueous solution either by physical methods or by the behavior of the acohols on oxidation.

The present chapter will be devoted essentially to ethyl alcohol and glycerol. The processes described for the quantitative determination of these compounds include the application of physical methods, methods of oxidation and methods of acetylation. In connection with the determination of ethyl alcohol, special methods for the detection or determination of a few of its more important homologues will be briefly outlined. For more extended discussions of the analytical chemistry of the alcohols the work of Allen ‡ should be consulted.

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* Analyse und Konstitutionsermittelung organischer Verbindungen, Berlin, 1903. Determination of Radicles in Carbon Compounds (English Edition by Tingle), New York, 1903.

† Identification of Pure Organic Compounds, New York, 1904.

‡ Commercial Organic Analysis, Volume I, Philadelphia, 1898.

## ETHYL ALCOHOL.

Pure ethyl alcohol is a colorless, mobile liquid of characteristic penetrating odor and "hot" pungent taste, boiling at about 78.4°. It mixes with water in all proportions and is only with great difficulty obtained in the anhydrous or "absolute" state. According to Allen the presence of as small a proportion as 0.5 per cent. of water in alcohol is indicated by the pink color assumed by the liquid on introducing a crystal of potassium permanganate. The so-called "absolute alcohol" used in analytical operations ordinarily contains from 0.2 to 1 per cent of water.

Until recently the density of anhydrous alcohol at 60° F. (15.55° C.) was considered to be 0.79381 referred to water at the same temperature. Squibb obtained alcohol which, while not absolutely anhydrous, had a density of only 0.79350 at 60° F. indicating that the alcohol previously considered absolute must have contained at least 0.1 per cent. of water.

On mixing alcohol with water a considerable evolution of heat takes place and the volume of the mixture after cooling is less than the sum of the volumes of alcohol and water mixed. This contraction is not uniformly proportional to the amount of alcohol in the mixture. Hence in mixtures of water and alcohol the relation between the percentages of alcohol "by volume" and "by weight" varies somewhat with the strength of the solutions. These variations together with the differences in the density of the supposedly absolute alcohol used as standard by the various observers, account for the small discrepancies found on comparing the commonly used tables showing the relation between density and percentage. Thus the table of Windisch which is usually given in German text-books, that of Allen which appears to be based on Hehner's table, and that of Edgar Richards based mainly on the work of Squibb, is each different, though the variations are rarely sufficient to be noticeable in ordinary work.

Alcohol dissolves many organic substances which are not soluble in water, but inorganic compounds insoluble in water are usually also insoluble in alcohol. As a rule chlorides, bromides, iodides, and acetates are soluble in fairly strong alcohol, while carbonates, borates, sulphates, phosphates, oxalates and tartrates are only very sparingly soluble.

## Detection and Identification.

### *Lieben's Iodoform Test.*

The "iodoform test" while not distinctive is often useful. It may be carried out as follows: To 10 c.c. of the clear liquid to be tested, add 5 or 6 drops of 10 per cent. solution of sodium or potassium hydroxide, heat to about 50° C. and add drop by drop with constant shaking a saturated solution of iodine in aqueous potassium iodide until the liquid becomes just permanently yellowish-brown, then carefully decolorize by adding more of the hydroxide solution, avoiding excess. If alcohol were present, iodoform gradually separates out as a yellow or yellowish-white crystalline deposit. Even when very little iodoform is produced its odor can usually be recognized. While the test is quite delicate, the appearance of a precipitate of iodoform does not prove the presence of ethyl alcohol since it may result from various other compounds, especially acetone, aldehydes and the propyl and butyl alcohols. If the original liquid contained carbohydrate or organic acid it should be neutralized, distilled and the first portion of the distillate used for the test.

### *Ethyl Dinitrobenzoate Test.*

As a specific test for ethyl alcohol Mulliken * recommends the preparation of Ethyl 3, 5-Dinitrobenzoate as follows:

Heat together gently in a three-inch test-tube held over a small flame, 0.15 gm. of 3, 5-dinitrobenzoic acid and 0.20 gm. of phosphorus pentachloride. When signs of chemical action are seen, remove the heat for a few seconds. Then heat again, boiling the liquefied mixture *very gently* for one minute. Pour out on a very small watchglass and allow to solidify. As soon as solidification occurs, remove the liquid phosphorus oxychloride, with which the crystalline mass is impregnated, by rubbing the latter between two small pieces of porous tile. Place the powder in a dry five or six-inch test-tube. Allow four drops of the alcohol (which must contain not more than about 10 per cent. of water) to fall upon it, and then stopper the tube tightly without delay. Immerse the lower part of the test-tube in water having a temperature of 75°–85°. Shake gently, and continue the heating for ten minutes.

To purify the ester produced in the reaction, crush with a stirring rod any hard lumps which may form when the mixture cools

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* *Loc. cit.*, p. 168.

and boil gently with 15 c.c. of methyl alcohol (2 : 1) until all is dissolved or for a minute or two. Filter boiling hot if the solution is not clear. Cool, shake and filter. Wash with 3 c.c. cold methyl alcohol (2 : 1). Recrystallize from 9 c.c. of boiling methyl alcohol (2 : 1). Wash with 2 c.c. of the same solvent. Spread out the product on a piece of tile. Allow to become air-dry, and determine the melting point.

Ethyl 3, 5-Dinitrobenzoate, the product of this test crystallizes in white needles melting at 92°–93° (uncorr.).

The corresponding derivatives of methyl, propyl, butyl, and isobutyl alcohols melt at 107.5° (uncorr.), 73°–73.5° (uncorr.), 64° (uncorr.) and 83°–83.5° (uncorr.) respectively.

### Determination by the Specific Gravity Method.

This method which is the most generally applicable and the most accurate for the determination of alcohol in ordinary alcoholic liquors, consists in obtaining the alcohol mixed only with water and determining the amount of alcohol present by finding the specific gravity of this aqueous solution. It will be convenient to describe first the method as applied to fermented liquors and then note the modifications to be introduced in the analysis of other alcohol preparations.

#### *Alcohol in Fermented Liquors.*

Dry and weigh a 100 c.c. graduated flask. Fill to the holding mark with the sample* and weigh. Transfer to a distilling flask of 250 to 300 c.c. capacity, rinsing out the measuring flask with about 50 c.c. of water. Add about one-tenth gram of calcium carbonate to neutralize any acid present, or carefully neutralize by means of dilute sodium or potassium hydroxide, connect with a well cooled condenser and distil, collecting the distillate in the flask used for measuring the sample. When the receiver is filled to the mark remove it, wipe the outside with a dry cloth and weigh. By means of a pyknometer determine accurately (to five decimal places) the specific gravity of the distillate at 15.55° C. (60° F.) compared with recently boiled distilled water at the same.

* If the sample contains much carbon dioxide this should be removed as far as possible in advance by shaking the liquor in a large flask at room temperature.

PERCENTAGE OF ALCOHOL BY WEIGHT AND BY VOLUME.

[Recalculated from the determinations of Gilpin, Drinkwater and Squibb, by EDGAR RICHARDS.]

| Specific Gravity at $\frac{60°}{60°}$ F. | Per Cent. Alcohol by Volume | Per Cent. Alcohol by Weight | Specific Gravity at $\frac{60°}{60°}$ F. | Per Cent. Alcohol by Volume. | Per Cent. Alcohol by Weight. | Specific Gravity at $\frac{60°}{60°}$ F. | Per Cent. Alcohol by Volume. | Per Cent. Alcohol by Weight. |
|---|---|---|---|---|---|---|---|---|
| 1.00000 | 0.00 | 0.00 | 0.99281 | 5.00 | 4.00 | 0.98660 | 10.00 | 8.04 |
| 0.99984 | .10 | .08 | 268 | .10 | .08 | 649 | .10 | .12 |
| 968 | .20 | .16 | 255 | .20 | .16 | 637 | .20 | .20 |
| 953 | .30 | .24 | 241 | .30 | .24 | 626 | .30 | .29 |
| 937 | .40 | .32 | 228 | .40 | .32 | 614 | .40 | .37 |
| .99923 | 0.50 | 0.40 | .99215 | 5.50 | 4.40 | .98603 | 10.50 | 8.45 |
| 907 | .60 | .48 | 202 | .60 | .48 | 592 | .60 | .53 |
| 892 | .70 | .56 | 189 | .70 | .56 | 580 | .70 | .61 |
| 877 | .80 | .64 | 175 | .80 | .64 | 569 | .80 | .70 |
| 861 | .90 | .71 | 162 | .90 | .72 | 557 | .90 | .78 |
| .99849 | 1.00 | 0.79 | .99149 | 6.00 | 4.80 | .98546 | 11.00 | 8.86 |
| 834 | .10 | .87 | 136 | .10 | .88 | 535 | .10 | .94 |
| 819 | .20 | .95 | 123 | .20 | .96 | 524 | .20 | 9.02 |
| 805 | .30 | 1.03 | 111 | .30 | 5.05 | 513 | .30 | .11 |
| 790 | .40 | .11 | 098 | .40 | .13 | 502 | .40 | .19 |
| .99775 | 1.50 | 1.19 | .99085 | 6.50 | 5.21 | .98491 | 11.50 | 9.27 |
| 760 | .60 | .27 | 072 | .60 | .29 | 479 | .60 | .35 |
| 745 | .70 | .35 | 059 | .70 | .37 | 468 | .70 | .43 |
| 731 | .80 | .43 | 047 | .80 | .45 | 457 | .80 | .51 |
| 716 | .90 | .51 | 034 | .90 | .53 | 446 | .90 | .59 |
| .99701 | 2.00 | 1.59 | .99021 | 7.00 | 5.61 | .98435 | 12.00 | 9.67 |
| 687 | .10 | .67 | 009 | .10 | .69 | 424 | .10 | .75 |
| 672 | .20 | .75 | .98996 | .20 | .77 | 413 | .20 | .83 |
| 658 | .30 | .83 | 984 | .30 | .86 | 402 | .30 | .92 |
| 643 | .40 | .91 | 971 | .40 | .94 | 391 | .40 | 10.00 |
| 0.99629 | 2.50 | 1.99 | .98959 | 7.50 | 6.02 | .98381 | 12.50 | 10.08 |
| 615 | .60 | 2.07 | 947 | .60 | .10 | 370 | .60 | .16 |
| 600 | .70 | .15 | 934 | .70 | .18 | 359 | .70 | .24 |
| 586 | .80 | .23 | 922 | .80 | .26 | 348 | .80 | .33 |
| 571 | .90 | .31 | 909 | .90 | .34 | 337 | .90 | .41 |
| .99557 | 3.00 | 2.39 | .98897 | 8.00 | 6.42 | .98326 | 13.00 | 10.49 |
| 543 | .10 | .47 | 885 | .10 | .50 | 315 | .10 | .57 |
| 529 | .20 | .55 | 873 | .20 | .58 | 305 | .20 | .65 |
| 515 | .30 | .64 | 861 | .30 | .67 | 294 | .30 | .74 |
| 501 | .40 | .72 | 849 | .40 | .75 | 283 | .40 | .82 |
| .99487 | 3.50 | 2.80 | .98837 | 8.50 | 6.83 | .98273 | 13.50 | 10.90 |
| 473 | .60 | .88 | 825 | .60 | .91 | 262 | .60 | .98 |
| 459 | .70 | .96 | 813 | .70 | .99 | 251 | .70 | 11.06 |
| 445 | .80 | 3.04 | 801 | .80 | 7.07 | 240 | .80 | .15 |
| 431 | .90 | .12 | 789 | .90 | .15 | 230 | .90 | .23 |
| .99417 | 4.00 | 3.20 | .98777 | 9.00 | 7.23 | .98219 | 14.00 | 11.31 |
| 403 | .10 | .28 | 765 | .10 | .31 | 209 | .10 | .39 |
| 390 | .20 | .36 | 754 | .20 | .39 | 198 | .20 | .47 |
| 376 | .30 | .44 | 742 | .30 | .48 | 188 | .30 | .56 |
| 363 | .40 | .52 | 730 | .40 | .56 | 177 | .40 | .64 |
| .99349 | 4.50 | 3.60 | .98719 | 9.50 | 7.64 | .98167 | 14.50 | 11.72 |
| 335 | .60 | .68 | 707 | .60 | .72 | 156 | .60 | .80 |
| 322 | .70 | .76 | 695 | .70 | .80 | 146 | .70 | .88 |
| 308 | .80 | .84 | 683 | .80 | .88 | 135 | .80 | .97 |
| 295 | .90 | .92 | 672 | .90 | .96 | 125 | .90 | 12.05 |

PERCENTAGE OF ALCOHOL BY WEIGHT AND BY VOLUME.—*Continued.*

[Recalculated from the determinations of Gilpin, Drinkwater and Squibb, by EDGAR RICHARDS.]

| Specific Gravity at 60° F. | Per Cent. Alcohol by Volume. | Per Cent. Alcohol by Weight. | Specific Gravity at 60° F. | Per Cent. Alcohol by Volume. | Per Cent. Alcohol by Weight. | Specific Gravity at 60° F. | Per Cent. Alcohol by Volume. | Per Cent. Alcohol by Weight. |
|---|---|---|---|---|---|---|---|---|
| 0.98114 | 15.00 | 12.13 | 0.97608 | 20.00 | 16.26 | 0.97097 | 25.00 | 20.43 |
| 104 | .10 | .21 | 598 | .10 | .34 | 086 | .10 | .51 |
| 093 | .20 | .29 | 588 | .20 | .42 | 076 | .20 | .60 |
| 083 | .30 | .38 | 578 | .30 | .51 | 065 | .30 | .68 |
| 073 | .40 | .46 | 568 | .40 | .59 | 055 | .40 | .77 |
| .98063 | 15.50 | 12.54 | .97558 | 20.50 | 16.67 | .97044 | 25.50 | 20.85 |
| 052 | .60 | .62 | 547 | .60 | .75 | 033 | .60 | .93 |
| 042 | .70 | .70 | 537 | .70 | .84 | 023 | .70 | 21.02 |
| 032 | .80 | .79 | 527 | .80 | .92 | 012 | .80 | .10 |
| 021 | .90 | .87 | 517 | .90 | 17.01 | 001 | .90 | .19 |
| .98011 | 16.00 | 12.95 | .97507 | 21.00 | 17.09 | .96991 | 26.00 | 21.27 |
| 001 | .10 | 13.03 | 497 | .10 | .17 | 980 | .10 | .35 |
| .97991 | .20 | .12 | 487 | .20 | .26 | 969 | .20 | .44 |
| 980 | .30 | .20 | 477 | .30 | .34 | 959 | .30 | .52 |
| 970 | .40 | .29 | 467 | .40 | .43 | 949 | .40 | .61 |
| .97960 | 16.50 | 13.37 | .97457 | 21.50 | 17.51 | .96937 | 26.50 | 21.69 |
| 950 | .60 | .45 | 446 | .60 | .59 | 926 | .60 | .77 |
| 940 | .70 | .53 | 436 | .70 | .67 | 915 | .70 | .86 |
| 929 | .80 | .62 | 426 | .80 | .76 | 905 | .80 | .94 |
| 919 | .90 | .70 | 416 | .90 | .84 | 894 | .90 | 22.03 |
| .97909 | 17.00 | 13.78 | .97406 | 22.00 | 17.92 | .96883 | 27.00 | 22.11 |
| 899 | .10 | .86 | 396 | .10 | 18.00 | 872 | .10 | .20 |
| 889 | .20 | .94 | 386 | .20 | .09 | 861 | .20 | .28 |
| 879 | .30 | 14.03 | 375 | .30 | .17 | 850 | .30 | .37 |
| 869 | .40 | .11 | 365 | .40 | .26 | 839 | .40 | .45 |
| .97859 | 17.50 | 14.19 | .97355 | 22.50 | 18.34 | .96828 | 27.50 | 22.54 |
| 848 | .60 | .27 | 345 | .60 | .42 | 816 | .60 | .62 |
| 838 | .70 | .35 | 335 | .70 | .51 | 805 | .70 | .71 |
| 828 | .80 | .44 | 324 | .80 | .59 | 794 | .80 | .79 |
| 818 | .90 | .52 | 314 | .90 | .68 | 783 | .90 | .88 |
| .97808 | 18.00 | 14.60 | .97304 | 23.00 | 18.76 | .96772 | 28.00 | 22.96 |
| 798 | .10 | .68 | 294 | .10 | .84 | 761 | .10 | .04 |
| 788 | .20 | .77 | 283 | .20 | .92 | 749 | .20 | .13 |
| 778 | .30 | .85 | 273 | .30 | 19.01 | 738 | .30 | .21 |
| 768 | .40 | .94 | 263 | .40 | .09 | 726 | .40 | .30 |
| .97758 | 18.50 | 15.02 | .97253 | 23.50 | 19.17 | .96715 | 28.50 | 23.38 |
| 748 | .60 | .10 | 242 | .60 | .25 | 704 | .60 | .47 |
| 738 | .70 | .18 | 232 | .70 | .34 | 692 | .70 | .55 |
| 728 | .80 | .27 | 222 | .80 | .42 | 681 | .80 | .64 |
| 718 | .90 | .38 | 211 | .90 | .51 | 669 | .90 | .72 |
| .97708 | 19.00 | 15.43 | .97201 | 24.00 | 19.59 | .96658 | 29.00 | 23.81 |
| 698 | .10 | .51 | 191 | .10 | .67 | 646 | .10 | .89 |
| 688 | .20 | .59 | 180 | .20 | .76 | 635 | .20 | .98 |
| 678 | .30 | .68 | 170 | .30 | .84 | 623 | .30 | 24.06 |
| 668 | .40 | .76 | 159 | .40 | .93 | 611 | .40 | .15 |
| .97658 | 19.50 | 15.84 | .97149 | 24.50 | 20.01 | .96600 | 29.50 | 24.23 |
| 648 | .60 | .93 | 139 | .60 | .09 | 587 | .60 | .32 |
| 638 | .70 | 16.01 | 128 | .70 | .18 | 576 | .70 | .40 |
| 628 | .80 | .09 | 118 | .80 | .26 | 564 | .80 | .49 |
| 618 | .90 | .18 | 107 | .90 | .35 | 553 | .90 | .57 |

temperature. Find in the accompanying table * the percentages of alcohol by weight and by volume corresponding to this specific gravity.

The percentage by weight of alcohol in the distillate, multiplied by the weight of the latter shows the actual weight of alcohol distilled over and this divided by the weight of sample taken, gives the percentage by weight of alcohol in the liquor. The percentage by volume is of course the same for the original sample as for the distillate, if both are measured in the same flask at the same temperature.

*Alcohol in Other Solutions.*

*Distilled Liquors.*—Take a smaller amount of the sample so as to have not over 25 grams of alcohol present. There should be no acid present to require neutralization. Dilute the portion taken for analysis to 150 c.c. in the distilling flask, distil until 100 c.c. have passed over and complete the determination as described.

*Cordials, bitters, etc.*, which are likely to contain high percentages of both alcohol and extractive matters, should be diluted if necessary and treated as described for fermented liquors.

*Preparations containing chloroform, ether, or essential oils*, may be treated as follows: † Dilute 25 c.c. of the sample with water to about 100 c.c. in a separatory funnel, add sodium chloride to saturation and then 50 to 80 c.c. of light petroleum distillate (boiling below 60°). Shake vigorously for 5 minutes, allow to stand for half an hour, draw off the lower layer into another separatory funnel, wash again in the same way with a small amount of the petroleum ether and then draw off into a distillation flask. Unite the petroleum ether layers, wash with two successive portions each 25 c.c. of water saturated with salt, adding these washings to the main solution. Neutralize, if necessary, and distil in the usual way.

*Notes on the Specific Gravity Method.*

When the distillation is carried out as above described, 100 c.c. being distilled from an original volume of about 150 c.c. containing not more than 25 grams of alcohol, the distillation may be carried on at a fairly rapid rate. There is no danger that the whole of the alcohol will not be distilled over, and if the condenser is kept

* Condensed from the tables adopted by the Association of Official Agricultural Chemists, Bulletins 46 and 65, Bur. Chem., U. S. Dept. Agriculture.

† Thorpe and Holmes: *Journ. Chem. Soc.*, 1903, 83, 314.

cooled to room temperature and is properly connected with the receiver, only traces of alcohol need be lost by evaporation. The delivery tube of the condenser may either extend well into the neck of the receiving flask or may be connected with it by means of an "adapter." If unusual precautions to avoid evaporation are desired the receiver may be closed with a stopper having one hole to admit the adapter and another fitted with a small reflux condenser, which can be rinsed with a few drops of water toward the end of the operation.

It is necessary to exercise great care in the determination of the specific gravity of the distillate, especially as regards the temperature at which the pyknometer is filled. If this is different from the temperature for which the table is constructed, each weighing must be separately reduced to standard temperature since alcohol and water do not expand at the same rate. The safest way, both in calibrating the pyknometer and in making the determination, is to cool the liquid to a little below 15.5°, fill, and allow the temperature to rise slowly until the proper point is reached, then adjust the pyknometer, wipe it dry on the outside and weigh. Never hold the *pyknometer in such a manner that the temperature of its contents* can be affected by the warmth of the hand. Find the weight of water which the pyknometer holds at 15.5° * and use this as divisor in estimating the specific gravity of the distillate. Empty and dry the pyknometer, fill with distillate at 15.5° and weigh. If both observations are made at 15.5°, the weight of distillate divided by that of water gives the desired specific gravity. If the pyknometer is filled with distillate at a few degrees above or below 15.5°, the weight must be divided by the weight of water at 15.5° to find the "apparent specific gravity", then correct this by the following empirical formula given by Allen† in which $D$ is the specific gravity at 15.5°, $D'$ the "apparent" specific gravity, and $d$ the difference (in degrees centigrade) between 15.5° and the temperature at which $D'$ was determined:

$$D = D' \pm d\left(0.00014 + \frac{1 - D'}{150}\right).$$

* If for any reason the water is slightly above or below 15.5°, note the temperature accurately, divide the weight of water found by the weight of 1 c.c. at that temperature and multiply by 0.999055. The following may be taken as the weight of 1 c.c. of water in grams at the temperatures given: 13°, 0.999410; 14°, 0.999278; 15°, 0.999134; 16°, 0.998976; 17°, 0.998808; 18°, 0.998629; 19°, 0.998438; 20°, 0.998235; 21°, 0.998023; 22°, 0.997801; 23°, 0.997568.

The correction is added to, or subtracted from, the "apparent specific gravity" according as the pyknometer was filled at a temperature above or below the standard.

The specific gravity of the distillate being known to five decimal places, its alcohol content by weight and by volume can be found to the second decimal place by intercalculation between the values given in the table.

*Pyknometers.* — In using the ordinary form of specific gravity bottle having a simple ground glass stopper with capillary bore, care must be taken that no minute bubbles of air are entrapped when the stopper is inserted. This may be largely avoided by grinding the lower end of the stopper either convex or concave. When a pyknometer of this form is allowed to stand at a higher temperature than that at which it has been filled, the expansion forces some of the liquid out upon the top of the stopper, whence it may be lost either mechanically or by evaporation. Since the temperature of the laboratory is usually considerably above 15.5°, the weighing should be made as promptly as possible. The Geissler pyknometer has a thermometer inserted in the ground glass stopper so that the temperature of the contents can be read directly at any time. A side tubulure of small bore provides for the overflow at constant volume and is fitted with a ground glass cap, so that when the adjustment has been made there is no danger of a loss of contents in case the pyknometer is allowed to stand for a time at a higher temperature. Squibbs' pyknometer * has a body like an ordinary specific gravity bottle, but the ground neck is fitted with a narrow graduated stem instead of a stopper. Adjustment is secured by removing small portions of liquid from this stem by means of a capillary pipette and narrow strips of blotting board. The construction of the instrument makes it easy to observe when the contents are at the same temperature as the surrounding medium, since as long as any expansion or contraction is taking place, the column of liquid will move up or down in the stem. This instrument, like that of Geissler, avoids all inconvenience from overflow during weighing. It is especially useful when one wishes to use the same pyknometer for determinations of specific gravity at different temperatures. Ostwald objects to any pyknometer having a tight stopper on the ground that "as the conical angle of the stopper must be small in order to get it to

* *Journ. Amer. Chem. Soc.*, 1897, 19, 111.

fit tight, a slight elastic deformation of the neck causes a considerable displacement of the stopper in the direction of its axis," thus introducing a source of error not easily avoided. He therefore recommends* a modification of the Spengel pyknometer which has now come into quite common use. With care in avoiding the sources of error which have been mentioned, any standard form of pyknometer should give satisfactory results.

### Determination by the Boiling Point Method.

In mixtures of alcohol and water containing no appreciable amount of other volatile substances and only small quantities of dissolved solids, the difference between the boiling point of the mixture and that of pure water under the same conditions, gives a measure of the percentage of alcohol present. For the rapid determination of alcohol on this principle, several forms of ebullioscope have been devised. The liquid to be tested is boiled under a reflux condenser while a thermometer bulb is fixed just above the surface of the liquid so as to be entirely surrounded by the vapor. The more common technical forms, such as those of Pohl and Kappeller, have scales reading percentage of alcohol instead of thermometer scales. Water is first boiled in the apparatus and the scale adjusted so that the mercury stands at zero. If then the water be removed and the sample introduced and brought to boiling under the same barometric conditions, the point reached by the mercury column shows the amount of alcohol present.

Wiley† uses a delicate differential thermometer with an apparatus similar to that employed for the determination of molecular weights by the boiling point method. Up to five per cent. of alcohol, the depression of the boiling point is said to be so regular that the results are entirely satisfactory for practical work. In the ebullioscopes bearing scales graduated in terms of alcohol, the variations in the boiling point curve at the higher percentages are, of course, allowed for. The boiling point method is very rapid and gives results sufficiently accurate for many purposes. Reference may be made to Vaubel‡ for a general discussion of methods based on the determination of the boiling point and to

*Ostwald: Physico-Chemical Measurements (translated by Walker), New York, 1894. The Sprengel-Ostwald pyknometer is also described by Getman: Laboratory Exercises in Physical Chemistry, New York, 1904.

†*Journ. Amer. Chem. Soc.*, 1896, 18, 1063.

‡Quantitative Bestimmung organischer Verbindungen, Berlin, 1902.

Freyer* for experimental results on the influence of dissolved solids in the ebullioscopic determination of alcohol.

### Determination by Oxidation.

Under suitable conditions ethyl alcohol can be quantitatively oxidized to acetic acid by means of potassium dichromate in the presence of sulphuric acid. The amount of alcohol can then be ascertained either by determining the amount of dichromate reduced † or by distilling and titrating the acetic acid formed.‡ The conditions of oxidation must be carefully regulated and as a rule the method is used only for the determination of very small amounts of alcohol, the specific gravity method being preferable for the examination of any but very dilute solutions. The alcohol must of course be separated by distillation from any other oxidizable matter before the oxidation method can be applied. A comparison of the results obtained by oxidation with those shown by the specific gravity method may be useful in demonstrating the presence of homologous alcohols.

### Detection and Determination of Homologous Alcohols.

#### *Methyl Alcohol.*

In many countries, "methylated spirit" (usually a mixture of 9 parts ethyl alcohol with 1 part commercial wood spirit) is exempt from the taxes levied upon other forms of alcohol. The former is therefore much cheaper and there is great inducement to substitute it for pure ethyl alcohol in the preparation of tinctures, etc., where its use is, however, highly objectionable on account of the toxicity of the methyl alcohol. Under such circumstances the detection of small amounts of methyl alcohol or wood spirit is often of importance. In the United States, "methylated spirit" is not exempt from taxation (1904) and delicate tests for its detection are therefore of much less practical importance. Methyl alcohol itself may be fraudulently substituted for ethyl alcohol but this would be profitable only when considerable quantities were involved and in such cases the presence of the methyl alcohol should be shown by quantitative methods.

* *Ztschr. angew. Chem.*, 1896, 654.

† Hehner: *Analyst*, 1887, **12**, 25. Benedict and Norris: *Journ. Amer. Chem. Soc.*, 1898, **20**, 293.

‡ Dupre: *Journ. Chem. Soc.*, 1867, **20**, 495.

Aqueous solutions of methyl alcohol have specific gravities so close to those of ethyl alcohol of the same strength that the total amount of the two alcohols can be determined by distilling, taking the specific gravity of the distillate and calculating the percentage from the ethyl alcohol table. This distillate may then be examined for methyl alcohol by one of the following methods.

*Color Reactions.*—The color reactions now commonly used * depend upon oxidation of the methyl alcohol to formaldehyde which is then detected by one of the tests described in the next chapter. These tests can be depended upon only when very carefully carried out with attention to the precautions given by Mulliken and Scudder, † while great care must also be exercised in the interpretation of the tests inasmuch as positive reactions for methyl alcohol have been obtained ‡ in the case of a number of genuine wines and brandies.

*The Iodide Method of Lam* § is based upon the fact that methyl iodide is considerably heavier than ethyl iodide, the former having at 15° a specific gravity of 2.2677, while that of the latter is 1.9444. The mixture of alcohols to be tested is treated with red phosphorus and iodine and the resulting iodides distilled, purified, dried and weighed. A determination of specific gravity shows the percentage of methyl iodide in the mixture and from this the percentage of methyl alcohol in the original sample. Ethyl alcohol is not completely transformed into iodide, but according to Lam the transformation of the methyl alcohol is quantitative.

*The Oxidation Method of Thorpe and Holmes* ‖ is simpler and more direct. It depends upon the oxidation of the mixture of ethyl and methyl alcohols under such conditions that the former is converted into acetic acid while the latter is completely oxidized to carbon dioxide and water. The total amount of alcohols having been determined as above, a part of the distillate is mixed with water in such proportions that 50 c.c. of the mixture shall contain not more than 1 gram of methyl alcohol nor more than 4 grams of ethyl and methyl alcohols together. Fifty c.c. of this mixture are introduced

* Mulliken and Scudder: *Amer. Chem. Journ.*, 1900, **24**, 444. Prescott: *Pharmaceutical Archives*, 1901, **4**, 86. Bulletin 65, Bur. Chem., U. S. Dept. Agriculture.

† *Loc. cit.*

‡ Trillat: *Compt. rend.*, 1899, **1**. Wolff: *Ztschr. Unters. Nahr.-Genussm.*, 1901, **4**, 391.

§ *Ztschr. angew. Chem.*, 1898, 125.

‖ *Journ. Chem. Soc.*, 1904, **85**, 1.

into a 300 c.c. flask having a tight stopper and fitted with a funnel and side tube, 20 grams of potassium dichromate and 80 c.c. of dilute sulphuric acid (1 : 4) added and the mixture allowed to remain for 18 hours. A further quantity of 10 grams of potassium dichromate and 50 c.c. of sulphuric acid mixed with an equal volume of water are now added, and the contents of the flask heated to the boiling point for about 10 minutes, the evolved carbon dioxide being swept out of the apparatus by a current of air and collected in weighed soda-lime tubes. Under these conditions each gram of ethyl alcohol was found to yield about 0.01 gram of carbon dioxide. The remaining carbon dioxide found is calculated as being derived from the complete oxidation of methyl alcohol. This method is used for the quantitative determination of methyl alcohol in the Government Laboratory, London.

### *Amyl Alcohols.*

The amyl alcohols are the principal constituents of fusel oil, and most of the methods proposed for the determination of fusel oil in distilled liquors are essentially attempts to estimate the amyl alcohols. In order to separate the amyl alcohols from the relatively large amounts of ethyl alcohol ordinarily present, advantage is taken of the fact that the former are much more soluble in chloroform than is the latter, so that on shaking a small amount of chloroform with a distillate containing about 30 per cent. of alcohol, practically all of the amyl alcohols and only a little of the ethyl alcohol pass into the chloroform layer. The amount of amyl alcohol removed by the chloroform is usually estimated either :

1. By measuring the increase in volume of the chloroform layer (Roese's method).

2. By oxidizing with potassium dichromate and determining the amount of valerianic acid produced (Marquardt's method).

Neither method gives very satisfactory results. The Association of Official Agricultural Chemists * has adopted the Roese method as carried out by Windisch. Allen † recommends a modification of the Marquardt method.

### METHODS OF STATING STRENGTH OF ALCOHOL SOLUTIONS.

The most satisfactory method of expressing the amount of alcohol in a solution is in terms of percentage by weight. The use of

* Bull. 65, Bur. Chem., U. S. Dept. Agriculture.

† Commercial Organic Analysis, Vol. I, p. 154.

the "percentage by volume" is, however, probably more common, while frequently the results of an analysis of alcoholic liquor are stated in grams of alcohol per 100 c.c. of sample.

For revenue purposes, both in the United States and Great Britain, the strength of alcoholic liquors is expressed in terms of "proof spirit," but the term has different meanings in the two countries. *American proof spirit* contains 50 per cent. of alcohol by volume, corresponding to about 42.7 per cent. by weight. *British proof spirit* is defined by Parliament as having such a density that at 57° F., thirteen volumes shall weigh the same as twelve volumes of water at the same temperature. This corresponds to about 49.2 per cent. by weight.

The U. S. Pharmacopeia authorizes the designation "*Absolute Alcohol*" for that which contains at least 99 per cent. by weight and "*Alcohol*" for that containing 91 per cent. by weight or 94 per cent. by volume.

"*Spirit*" of the German Pharmacopeia contains about 86 per cent. of alcohol by weight.

"*Rectified Spirit*" of the British Pharmocopeia has 84 per cent. by weight and British "*Methylated Spirit*" consists of nine parts of rectified spirit to one part of commercial "wood spirit" or "wood naphtha," the latter containing, according to recent observations of Thorpe and Holmes, from 72 to 80 per cent. of methyl alcohol by volume.

## GLYCEROL.

Glycerol is a colorless, odorless, viscous liquid of sweet taste and neutral reaction, miscible in all proportions with water and with alcohol. It also dissolves in mixtures of alcohol and ether, but is only very sparingly soluble in pure ether * and practically insoluble in chloroform, carbon disulphide and benzene. The specific gravity of pure glycerol at 15° referred to water at the same temperature, is variously stated at from 1.265 to 1.2677. Anhydrous glycerol boils at about 290°, but evaporates rapidly at lower temperatures (160° or over) and the evaporation is greatly accelerated by the presence of a small amount of water. When kindled, glycerol burns with a blue flame and leaves no carbonaceous residue.

These properties together with the fact that it yields acrolein when heated with acid potassium sulphate, are usually sufficient for

* According to Lewkowitsch, one part of glycerol of 1.23 sp. gr. dissolves in about 500 parts of ether.

the identification of glycerol when in a fairly pure and concentrated state.

Glycerol is a good solvent for many substances, both organic and inorganic, and its presence often increases their solubility in aqueous and alcoholic solutions. This fact and the difficulty of distilling without loss * make it troublesome to separate glycerol as a pure aqueous solution as is done in the determination of alcohol. The method to be followed in removing the other substances which may interfere with the determination will depend upon the nature of the sample. The final determination is usually made in one of three ways: (1) By oxidation with potassium dichromate. (2) By the formation of triacetin. (3) By separation and weighing.

The first and second methods will be described as applied to crude or commercial glycerins, the third as applied to wines.

### Determination by Oxidation.

While other methods of determining glycerol by oxidation have been proposed, that of Hehner † is the one now generally used. It is based on the fact that glycerol can be quantitatively oxidized to carbon dioxide and water by heating with potassium dichromate in the presence of sulphuric acid.

*Reagents.* — (1) Strong solution of potassium dichromate made by dissolving 74.64 grams of the pure salt (previously pulverized and dried at 130°–135°) and diluting the solution to one liter. (2) Weak solution of dichromate made by diluting 100 c.c. of the strong solution to one liter. (3) A solution containing about 240 grams of ferrous ammonium sulphate and about 10 c.c. of concentrated sulphuric acid per liter.

*Apparatus* which is to come in contact with the dichromate solution must be very carefully cleaned in advance with a solution of dichromate in strong sulphuric acid.

*Determination.* — Weigh such an amount of the sample as will contain about 1.5 grams of actual glycerol into a 100 c.c. flask, dilute to about 10 c.c., add 0.5 to 1.0 gram silver oxide and let

* An approximate separation of glycerol from dissolved solids can easily be made by distilling with steam. At sufficiently reduced pressure the steam distillation may be carried on with very little loss of glycerol, this process being used on a large scale as a means of refining commercial glycerin. The necessary apparatus is, however, not always available in the laboratory and the completeness of distillation and recovery of the glycerol has not yet been definitely settled.

† *Journ. Soc. Chem. Ind.*, 1889, 8, 4.

stand for ten minutes, shaking frequently; then add basic lead acetate in slight excess and fill to the mark. After thorough shaking, filter through dry paper, discarding the first few c.c.; transfer 25 c.c. of the filtrate to beaker, add 30 c.c. of a mixture of equal volumes of sulphuric acid and water and then run in 50 c.c. of the stronger solution of potassium dichromate, measuring the volume with the greatest possible accuracy. Cover the beaker with a watch glass and place it in boiling water for two hours. During this time the exact strength of the ferrous sulphate solution is determined by titration against the dichromate. At the end of the two hours remove the beaker from the boiling water, add 100 c.c. water and titrate the excess of dichromate remaining. Each cubic centimeter of the stronger dichromate solution which has been reduced indicates the oxidation to carbon dioxide and water of 0.01 gram of glycerol.

*Notes.*—The treatment of the sample with silver oxide precipitates chlorides and oxidizes any aldehydes which may be present. Basic lead acetate precipitates proteids, resinous matter, and the higher fatty acids. Hehner supposed that these two reagents would remove all impurities likely to be found in crude glycerins which could be oxidized by dichromate under the conditions given. In a number of cases, however, genuine crude glycerins have given high results by this method, doubtless because they contained oxidizable impurities not removed by silver oxide and basic lead acetate.

As the oxidizing dichromate solution is a strong one, it must not only be measured with the greatest care but it should always be shaken before using in order to wash down any drops of water which may have evaporated from the solution and condensed on the inner surface of the bottle. Attention must also be paid to the temperature at the time of measuring. According to Hehner, this solution expands 0.05 per cent. for each increase of one degree C.

As the method is ordinarly described, the excess of dichromate remaining after the oxidation of the glycerol is reduced by adding a known amount of the ferrous ammonium sulphate solution and the excess of the latter titrated by the weaker dichromate. The green color of the solution makes the end reaction with ferricyanide somewhat difficult to detect and it will usually be found more satisfactory to determine the excess of the stronger dichromate by direct titration with the ferrous solution. Then if the end point

is accidentally overrun, add quickly 5 or 10 c.c. of the weak (equal to 0.5 or 1.0 c.c. of the strong) dichromate and continue titrating with the ferrous solution until the blue reaction with ferricyanide appears.

DETERMINATION BY ACETYLATION.

On heating with acetic anhydride, glycerol is completely converted into the triacetate. This is the basis of the acetin method of Benedict and Cantor * which is applied to commercial glycerins as follows:

Weigh 1 to 1.5 grams of the sample (which must not contain more than 30 to 40 per cent. of moisture) into a flask, add 3 to 5 grams of recently fused sodium acetate and 7 to 10 c.c. acetic anhydride and boil under a reflux condenser for one and one-half hours. Allow to cool, pour 50 c.c. of water into the flask through the condenser and heat gently with the condenser still attached until the liquid begins to boil and the oily layer of triacetate goes into solution. Remove the condenser, add phenolphthalein and carefully neutralize with 5 per cent. sodium hydroxide solution. Care must be taken to avoid any excess of alkali at this point as the triacetate is easily hydrolyzed. Shake the solution well to avoid any local excess of alkali during the neutralization, and stop adding alkali as soon as the solution changes to a reddish-yellow color. If the solution were made sufficiently alkaline at this point to show a full pink color, the final result would be too low.

To the neutralized solution add 25 c.c. of a 10 per cent. solution of sodium hydroxide (accurately measured by a pipette), boil for 10 to 15 minutes and titrate the excess of alkali with accurately standardized acid. At the same time determine the exact strength of the alkali solution by measuring out another portion of 25 c.c. with the same pipette and titrating with the same acid, using phenolphthalein as indicator. The difference between the two titrations shows the amount of alkali consumed in saponifying the triacetate and from this the amount of glycerol is calculated, one molecule of glycerol being equivalent to three molecules of sodium hydroxide.

*Notes.* — If sufficient care is taken to prevent any of the acetin from being lost by evaporation or saponified during the neutralization of the excess of acetic anhydride, the results by this method are very accurate. If these precautions are not carefully observed

* *Ztschr. angew. Chem.*, 1888, 460.

the results are likely to be somewhat low. Practically pure glycerol should give the same results by acetylation as by oxidation. Crude glycerins often give much higher results by the latter method. In such cases the acetin method should be repeated with special attention to the precautions already mentioned and to the moisture present in the sample and the sodium acetate used. Unless the sample contains over 60 per cent. of glycerol and the sodium acetate is strictly anhydrous, the formation of the triacetate is likely to be incomplete. When obtained under the proper conditions, the results by the acetin method should be preferred to those by the dichromate because the impurities of crude glycerins are more likely to vitiate the latter than the former method. In cases where other oxidizable substances are absent or can be entirely removed, it is a good plan to make a determination by each method and average the results obtained. For dilute solutions the dichromate method, if applicable, is much more convenient because the acetin method cannot be applied until after the glycerol has been concentrated by careful evaporation.

### Determination by Separation and Weighing.

For the estimation of glycerol in fermented liquors where it occurs mixed with relatively large amounts of alcohol, sugar, organic acids and resinous, nitrogenous and other "extractive" matters, the plan usually followed is to evaporate off the greater part of the alcohol and water and then treat with lime and after further evaporaton take up the glycerol with strong alcohol, leaving the greater part of the extractive matters in the residue. The substances, other than glycerol, which pass into the alcohol solution are largely precipitated by the addition of ether and the alcohol-ether solution is then evaporated.

The details of the process as adopted by the Association of Official Agricultural Chemists * for the determination of glycerol in wine are as follows:

Evaporate 100 c.c. of wine † in a porcelain dish on the water

* Bull. 65, Bur. Chem., U. S. Dept. Agriculture.

† With wines whose extract exceeds 5 grams per 100 c.c., heat to boiling in a flask the portion to be used in the determination of glycerol, and treat with successive small portions of milk of lime until it becomes, first, darker, and then light in color. When cool, add 200 c.c. of 96 per cent. alcohol (sp. gr. 0.812), allow the precipitate to subside, filter, and wash with 96 per cent. alcohol. Evaporate the filtrate to about 10 c.c., add about 5 grams of sand and from 1.5 to 2 c.c. of milk of lime, and proceed as directed above.

bath to a volume of about 10 c.c. and treat the residue with about 5 grams of fine sand and with from 1.5 to 2 c.c. of milk of lime (containing about 40 per cent. of calcium hydroxide or 30 per cent. of calcium oxide) for each gram of extract present, and evaporate almost to dryness. Treat the moist residue with 5 c.c. of 96 per cent. alcohol (sp. gr. 0.812), remove the substance adhering to the sides of the dish with a spatula, and rub the whole mass to a paste, with the addition of a little more alcohol. Heat the mixture on the water bath, with constant stirring, to incipient boiling, and decant the liquid into a flask graduated at 100 and 110 c.c. Wash the residue repeatedly by decantation with 10 c.c. portions of hot 96 per cent. alcohol. Cool the contents of the flask to 15°, dilute to the 110 c.c. mark with 96 per cent. alcohol, and filter through a folded filter. Evaporate 100 c.c. of the filtrate to a sirupy consistency in a porcelain dish on a hot, but not boiling, water bath, transfer the residue to a small glass-stoppered graduated cylinder with 20 c.c. of absolute alcohol, and add three portions of 10 c.c. each of absolute ether, mixing after each addition. Let stand until clear, then pour off through a filter, and wash the cylinder and filter with a mixture of one part absolute alcohol to one and one-half parts of absolute ether, pouring the wash liquor also through the filter. Evaporate the filtrate to a sirupy consistency, dry for one hour at the temperature of boiling water, weigh, ignite, and weigh again. The loss on ignition increased by one-tenth gives the glycerol expressed in grams per 100 c.c.

*Notes.* — When a mixture of glycerol and water is concentrated at about the boiling point of the latter, water evaporates without any considerable loss of glycerol until the latter becomes quite highly concentrated. The point at which glycerol begins to be lost depends upon a number of conditions such as temperature, the depth of the layer, etc., but some loss always occurs in expelling the last of the water. The same is true with regard to the concentration of alcoholic solutions though the loss here is less as glycerol is less volatile with alcohol than with water vapor. On the other hand the glycerol obtained by this method has other impurities than ash, and these are calculated as glycerol. For detailed tests of the method see the work of Neubauer and Borgmann on wines * and that of Clausnitzer on beers.†

* *Ztschr. anal. Chem.*, 1878, 17, 442.

† *Ibid.*, 1881, 20, 58.

See also — Friedeberg: Ueber Glycerinbestimmung in vergohrenen Getränken, Dis-

### Examination of Commercial Glycerol.

Commercial glycerol (or "glycerin") always contains water, and all but the best "chemically pure glycerol" contains appreciable amounts of impurities derived from the original fat or from the saponifying agents. The tests to be applied and the allowable limits of impurity will depend upon the purpose for which the glycerin is to be used. The quantitative determinations most commonly required are: specific gravity, carbonaceous residue, ash and salt. The higher grades of glycerin must also be practically neutral and show no turbidity due to fatty acids when the sample is diluted with water, and hydrochloric acid added.

Additional tests and specifications for pharmaceutical glycerin will be found in the *U. S. Pharmacopeia* and *Dispensatory*.

Glycerin used for the manufacture of nitroglycerol need not be colorless but must have a high degree of purity as shown by the following quantitative determinations.

#### *Specific Gravity.*

The viscosity of glycerol and the fact that when highly concentrated it is quite hygroscopic make it necessary to use the greatest care in this determination. Hehner * recommends the Sprengel tube and Lewkowitsch † the ordinary specific gravity bottle. It is usually more convenient to take the specific gravity by using the hydrostatic sinker with the analytical balance. For this purpose the Reimann sinker graduated for 15° is very convenient. The glass body encloses a thermometer (usually graduated from 0° to 27°) and is of such size that when immersed it displaces 5 (or sometimes 10) grams of water at 15°. This calibration should be tested for each sinker as it is received from the dealer by suspending it over the pan of the analytical balance, finding its weight in air and then determining its weight when suspended in the same position but immersed in recently boiled distilled water at 15°. This is easily done by placing a bridge over the balance pan and on this bridge a small cylinder containing enough water to cover the sinker to a depth of about 1 cm. The sinker should hang in the center of the cylinder and the diameter of the latter should

sertation, Berlin, 1890. Schaumann: Bestimmung von Glycerin im Wein, Dissertation, Erlangen, 1891.

* *Journ. Soc. Chem. Ind.*, 1889, 8, 4.

† Oils, Fats and Waxes [3d Ed.], p. 1104.

be about three times that of the former. Having found the weight of water displaced, remove and dry the sinker, replace the cylinder of water by one filled to the same depth with the sample of glycerin to be tested and weigh the sinker suspended in the glycerin at 15°. The loss of weight in glycerin divided by that in water shows the specific gravity of the former referred to water at the same temperature.

If the sample consists of glycerol and water with only insignificant amounts of impurities, the specific gravity serves as a measure of the concentration. This method is, however, considerably less accurate than in the determination of alcohol because the specific gravity of pure anhydrous glycerol is less accurately known than that of absolute alcohol. The most generally accepted tables of specific gravities of glycerol solutions are those of Lenz * and Nicol.† Reduced to 15.5° and 15°, the values for concentrated solutions as found by these observers are:

| Glycerol Percent. | Specific Gravity at 15.5° Lenz. | Specific Gravity at 15° Nicol. | Glycerol Percent. | Specific Gravity at 15.5° Lenz. | Specific Gravity at 15° Nicol. |
|---|---|---|---|---|---|
| 100 | 1.2674 | 1.26596 | 87 | 1.2327 | 1.23128 |
| 99 | 1.2647 | 1.26335 | 86 | 1.2301 | 1.22855 |
| 98 | 1.2620 | 1.26072 | 85 | 1.2274 | 1.22583 |
| 97 | 1.2594 | 1.25809 | 84 | 1.2248 | 1.22310 |
| 96 | 1.2567 | 1.25547 | 83 | 1.2222 | 1.22038 |
| 95 | 1.2540 | 1.25285 | 82 | 1.2196 | 1.21766 |
| 94 | 1.2513 | 1.25021 | 81 | 1.2169 | 1.21493 |
| 93 | 1.2486 | 1.24756 | 80 | 1.2143 | 1.21221 |
| 92 | 1.2460 | 1.24487 | 79 | 1.2117 | 1.20949 |
| 91 | 1.2433 | 1.24217 | 78 | 1.2090 | 1.20677 |
| 90 | 1.2406 | 1.23945 | 77 | 1.2064 | 1.20404 |
| 89 | 1.2380 | 1.23673 | 76 | 1.2037 | 1.20131 |
| 88 | 1.2353 | 1.23400 | 75 | 1.2011 | 1.19857 |

It has also been proposed to apply a correction for the influence of salt on the specific gravity and use the corrected figure as a rough measure of the glycerol present in crude glycerins.‡

If the percentage of glycerol indicated by the specific gravity is less than that shown by the dichromate method, the presence of oxidizable compounds of lower density than glycerol is indicated. Thus a sample of "light" glycerin examined by Noyes and Watkins § was found to contain about 38 per cent. of trimethylene

* Recommended by Hehner, *Journ. Soc. Chem. Ind.*, 1889, 8, 4.

† U. S. Dispensatory (18th Ed.), p. 657.

‡ Richardson and Joffe, *Journ. Soc. Chem. Ind.*, 1898, 17, 330.

§ *Journ. Amer. Chem. Soc.*, 1895, 17, 890.

glycol. Since this glycerin was obtained largely from refuse house fat, the glycol was probably formed by spontaneous saponification and subsequent fermentation of the glycerol in the impure rancid fat. This glycol has also been found in considerable quantity in the "tank liquor" separating from the fat before saponification* and is probably not an uncommon impurity, especially in glycerin obtained from refuse fats.

Samples containing glycol would, of course, yield lower results by the acetin than by the dichromate method.

### *Carbonaceous Residue and Ash.*

Weigh about 50 grams of glycerin into a 50 c.c. platinum dish, heat cautiously over a Bunsen burner until the sample ignites, remove the burner and allow the glycerol to burn away, being careful that the dish is not exposed to draughts. When burning ceases, transfer the dish at once to a desiccator and when cool weigh quickly, as the residue is apt to be hygroscopic. After weighing the carbonaceous residue, burn to whiteness and weigh the ash.

### *Sodium Chloride.*

Weigh 50 to 100 grams of sample, dilute with twice its volume of water, add a few drops of potassium chromate solution and titrate with standard silver nitrate, preferably tenth-normal or of such strength as to be equivalent to a one per cent. solution of sodium chloride.

### *Specifications for Dynamite Glycerin.*

The following specifications are furnished by the firm of Wm. F. Jobbins as representing the requirements of a majority of the nitroglycerol manufacturers in the United States at the present time (1904).

*Specific Gravity* at 15°, not less than 1.2625.

*Carbonaceous Residue* (including ash) and *Ash* as determined by the method given above, must not exceed 0.03 per cent. and 0.01 per cent. respectively.

*Sodium Chloride* determined as above must not exceed 0.01 per cent. and is usually very much less than this amount.

*Total Acid Equivalent* ("*Fatty Acids*") is determined as follows: Dilute the sample with twice its volume of water, add a few drops of phenolphthalein solution and a decided excess of standard

* Twitchell: *Journ. Amer. Chem. Soc.*, 1895, 17, 891.

sodium hydroxide; boil five minutes and then titrate the excess of alkali by standard acid. The glycerin must not consume more than 0.10 per cent. of its weight of sodium hydroxide in this test.

For a fuller discussion of the examination of "dynamite glycerin," see a paper by Barton in the *Journal of the American Chemical Society* (1895, 17, 277).

Detailed information on the testing of "dynamite glycerin" and other grades of commercial glycerol will be found in Lewkowitsch's "Oils, Fats and Waxes," and in Allen's "Commercial Organic Analysis."

## CHAPTER IV.

### Aldehydes.

The most important methods for the detection and determination of aldehydes are based upon reactions of oxidation, of condensation and of direct addition.

The readiness with which aldehydes undergo oxidation gives them the property *of reducing ammoniacal silver solution*, which is the basis of one of the most delicate qualitative tests for this group of compounds. The test may be carried out as follows: *

Mix, in a test-tube previously cleaned with hot sodium hydroxide solution, 1 c. c. of ammoniacal silver nitrate solution (containing one part of silver nitrate in ten parts of ammonium hydroxide of 0.923 sp. gr.) and 1 c.c. of ten per cent. sodium hydroxide solution. Shake the mixture in the tube and then allow two or three drops of the solution to be tested to flow slowly down the moistened glass surface into the reagent. Shake and allow to stand cold for five minutes. Aldehydes (and a few other compounds including some of the polyatomic alcohols) cause the production of a dark brown or black precipitate or mirror of metallic silver. This reaction is given by all of the ordinary aldehydes of the fatty series, including the aldose carbohydrates, but not by all aromatic aldehydes.

The ammoniacal silver solution and the sodium hydroxide must not be mixed in advance and must always be kept cool as a dangerously explosive precipitate is apt to form on warming or on long standing. The use of a mixture of sodium hydroxide and ammoniacal silver nitrate (Tollens' aldehyde reagent) makes the test more delicate than when the ammoniacal silver solution is used alone.

Alkaline solutions of other metals are reduced by many aldehydes, especially on boiling, and many quantitative methods for individual aldehydes are based upon the determination of the amount of metal reduced.

*Condensation reactions, especially with* phenylhydrazine, hydrox-

* Noyes and Mulliken: Identification and Class Reactions of Organic Substances. Mulliken: Identification of Pure Organic Compounds, Vol. I., p. 22.

ylamine and phenols, are often used for the detection and sometimes for the determination of aldehydes. A general discussion of such methods will be found in the works of Vaubel * and of Meyer. † Several special methods will be described in this and the two following chapters.

Of the addition reactions of aldehydes, that with bisulphite is of especially wide application. On shaking a liquid aldehyde or a concentrated solution of aldehyde in water or ether, with an equal volume of strong sodium bisulphite solution, addition takes place with the formation of the saturated compound $RCH(OH)SO_3Na$ which usually separates as a white crystalline precipitate. Ketones containing the $CH_3CO$ group also give the reaction. A negative result is not conclusive as the addition product may be too soluble to appear as a precipitate.

According to Ripper,‡ the bisulphite reaction can be utilized for the determination of any aldehyde soluble in water or which can be brought into solution by a small amount of alcohol. A one-half per cent. solution of the aldehyde is mixed with twice its volume of a solution of potassium bisulphite of known strength (about 12 grams per liter), and after 15 minutes the excess of bisulphite is determined by titration with iodine. Ripper applied this method with satisfactory results to solutions of formaldehyde, acetaldehyde, benzaldehyde and vanillin.

A similar addition reaction gives rise to the well-known and delicate "fuchsin test" for aldehydes. This test, as developed by Mulliken,§ is as follows:

To prepare the fuchsin aldehyde reagent, dissolve 0.2 gram of rosanilin, or, if the free base cannot be obtained, of the hydrochloride or acetate in 10 c.c. of a freshly prepared, cold, saturated aqueous solution of sulphur dioxide. Allow the solution to stand until all signs of pink disappear and it becomes colorless or pale yellow. This will require several hours. Then dilute with water to 200 c.c. and preserve for use in a tightly-stoppered bottle.

To 5 c.c. of this reagent add 0.05 gram, or one drop, of the substance to be tested (if pure, or a few drops if in solution). If the substance is a liquid, or dissolves in the reagent, allow to stand two

* Bestimmung organische Verbindungen.

† Analyse und Konstitutionsermittlung organischer Verbindungen.

‡ *Monatsh. Chem.*, 1900, 21, 1079.

§ Identification of Pure Organic Compounds, Vol. I, p. 15.

minutes and observe the color. If the substance does not dissolve, shake gently for two minutes and then observe the color. The appearance of a distinct pink, red, purple or blue coloration indicates the presence of an aldehyde. The test to be of value must be applied under carefully regulated conditions. The reagent is reddened by alkalies or alkaline salts of weak acids, by heating or by long exposure to air at ordinary temperature. In general, the test as here described distinguishes aldehydes other than carbohydrates from the latter and from ketones. A few acetals show the reaction through being partially hydrolyzed to aldehydes under the conditions of the test. Acetone and some other soluble ketones prepared by destructive distillation gradually redden the reagent if added to it in large quantity or if allowed to remain in contact for a number of minutes; but this is thought to be due chiefly, if not wholly, to the presence of traces of aldehydes or acetals (Mulliken).

This reaction serves for the detection of minute quantities of aldehydes present as impurities in commercial alcohol, and for the colorimetric estimation of aldehydes in distilled liquors.*

## FORMALDEHYDE.

Formaldehyde gas, produced by the partial oxidation of methyl alcohol, is freely soluble in water and is most commonly handled as a 35 to 40 per cent. aqueous solution. Such solutions are often designated formalin, formol or formal. More dilute solutions are extensively sold as food preservatives, often under fanciful or misleading names.

In dilute aqueous solution, formaldehyde exists in the "monomolecular" state, as $CH_2O$. Such solutions do not change if kept at ordinary temperature in closed vessels. When an aqueous solution is concentrated either by spontaneous evaporation or by heating, a white flocculent deposit appears. If the solution is then separated from the deposit it is found to contain condensed or polymerized formaldehyde.† The material which deposits from a concentrated aqueous solution of formaldehyde has, after drying,

*Medicus: *Forschungsber. über Lebensmittel*, 1895, 1, 299; Bulls. 46 and 65, Bur. Chem., U. S. Dept. Agriculture.

† Tollens and Mayer: *Ber. deut. chem. Ges.*, 1888, 21, 1571, 3503. Kraut, Eschweiler and Grossmann: *Ann. Chem.*, 1890, 258, 103.

the composition $(CH_2O)_6 \cdot H_2O$ to $(CH_2O)_8 \cdot H_2O$.* It is amorphous, soluble in warm water and has an odor resembling that of formaldehyde. The paraformaldehyde of commerce consists essentially of this material.

Metaformaldehyde (oxymethylene, "trioxymethylene"), $(CH_2O)_x$, may also be formed by evaporation of formaldehyde solutions. By prolonged digestion at ordinary temperature or by heating for a short time at 130°–150° with a large excess of water, metaformaldehyde passes into solution and into the "mono-molecular" form. Polymeric modifications of formaldehyde in aqueous solution resemble closely the original substance in its behavior toward reagents so that, as measured by the ordinary methods, a solution does not lose strength by the partial polymerization of the formaldehyde so long as all remains in solution.

Commercial solutions of formaldehyde commonly contain methyl alcohol and may contain any of the impurities of commercial wood spirit. Solutions of the usual strength, 35 to 40 per cent., should have specific gravities of about 1.08 to 1.11 at 15°, lower figures ordinarily indicating the presence of excessive amounts of methyl alcohol.†

The methods given in this chapter for the detection and determination of formaldehyde refer especially to the examination of commercial solutions containing only such impurities as ordinarily occur in crude preparations of formaldehyde, or substances which might be used with formaldehyde in preservative mixtures. The examination of food products for formaldehyde will be discussed in connection with other food preservatives in a subsequent chapter.

If a solution to be examined contains dissolved solids which interfere with the direct application of the tests as described, it can be acidified with a small excess of phosphoric or sulphuric acid, distilled, and the test applied to the distillate. The latter, however, will never contain all of the formaldehyde, since some is always polymerized and left as paraformaldehyde in the distilling flask.

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* Lösekann: *Chem. Ztg.*, 1890, 14, 1408. Delephine: *Compt. rend.*, 1897, 124, 1525; Beilstein: Organische Chemie, Ergänzbd., I., 467.

† On the determination of methyl alcohol in formaldehyde solutions see—Duyk: *Ann. Chim. Anal. Appl.*, 1901, 6, 407; *Journ. Chem. Soc.*, 1902, 82, ii, 110. Stritrar: *Ztschr. anal. Chem.*, 1904, 43, 401. Gnehm and Kaufler: *Ztschr. angew. Chem.*, 1904, 17, 673; 1905, 18, 93. Bamberger: *Ibid.*, 1904, 17, 1246.

## Detection and Identification.

### *Resorcin Test.**

Mix one drop of a 1 per cent. aqueous solution of resorcin with 1 c.c. of a dilute aqueous solution (preferably about 0.2 per cent.) of the aldehyde. Allow the mixture to flow gently down the side of an inclined test-tube containing 3–5 c.c. of pure concentrated sulphuric acid (or incline the test-tube containing the mixture and pour in the acid). Impart a gentle rotary motion to the liquids by cautiously swaying the lower end of the tube through a circle about a decimeter in diameter, in such a manner as not to cause the disappearance of the two layers. If formaldehyde is present, a red ring slightly tinged with violet will soon appear. Above this ring a light flocculent precipitate, at first nearly white on its upper surface and red-violet beneath, but soon changing to flocks that are red throughout, will be seen suspended in the aqueous upper layer.

This reaction is very satisfactory for solutions containing one part of formaldehyde in 100 to 5000 parts of solution and can be detected to a dilution of 1 : 100,000. A similar reaction is obtained if phenol is used instead of resorcin.

### *Gallic Acid Test.* †

Mix 0.2 c.c. of a saturated solution of gallic acid in pure ethyl alcohol, with 1 to 2 c.c. of the solution to be tested and introduce a layer of concentrated sulphuric acid, as in the resorcin test. In the presence of formaldehyde, a green zone appears at the line of contact of the two liquids. This gradually changes to a pure blue ring, which, in the case of pure aqueous solutions of formaldehyde, can be detected without difficulty at a dilution of 1 : 500,000. If the solution tested contains as much as one part of formaldehyde in 20,000, a yellowish color appears immediately at the line of contact of the two liquids. This quickly turns green, and the blue color develops both above and below the green zone. If other substances which give color reactions are also present, the upper layer will vary in color, but the green and lower blue ring will still appear beneath

* Mulliken and Scudder: *Amer. Chem. Journ.*, 1900, 24, 451. Mulliken: Identification of Pure Organic Compounds, Vol. I, p. 24.

† Barbier and Jandrier: *Ann. Chim. Anal. Appl.*, 1, 325; Abs. *Analyst*, 1896, 21, 295. Mulliken and Scudder: *Amer. Chem. Journ.*, 1900, 24, 446.

(Mulliken and Scudder). On swaying the tube, or allowing it to stand for some time, the blue color spreads throughout the zone and a pure blue ring is usually obtained. The color is quite permanent and apparently quite characteristic, no other substance having been noted as giving the blue ring. Acetaldehyde tested in the same way gives a reddish brown coloration.

### *Hydrochloric Acid and Casein Test.* *

Mix 5 c.c. of the solution to be tested with 5 c.c. of pure milk in a porcelain casserole, add 10 c.c. of concentrated hydrochloric acid containing 0.002 gram of ferric chloride and heat slowly over a free flame nearly to boiling, meanwhile giving the casserole a rotary motion to break up the curd. A violet coloration indicates formaldehyde. According to Leach, various aldehydes give color reactions under this treatment, but formaldehyde alone shows the unmistakable, violet coloration. This test is especially useful for the detection of formaldehyde in milk, and will be more fully discussed in that connection.

### *Methylene-di-β-naphthol Test.*

Since formaldehyde is frequently sold under other names, its identification by some method independent of the above color reactions may be a matter of importance. In such cases the following test given by Mulliken † will be useful.

Place in a test-tube 3 drops of a 30 to 40 per cent., or 10 drops of a 10 per cent. solution, 3 c.c. of dilute alcohol (1 : 2), 0.04 to 0.06 gram β-naphthol, and 3 to 5 drops of concentrated hydrochloric acid. Boil gently until the liquid becomes filled with an abundant precipitate of small white needles. Filter while hot. Wash with 1 c.c. of dilute alcohol (1 : 2). Boil the precipitate with 4 c.c. of dilute alcohol (1 :1.) (It is not necessary that all should dissolve ) Cool and filter off the precipitate. Wash with 1 c.c. of dilute alcohol (1 : 1.) Dry on porous tile in a warm place and determine the melting point.

Methylene-di-β-naphthol, the product, forms white needles which, when the temperature in the neighborhood of the melting point is raised at the rate of 1° in 15 seconds, begin to turn brown

* Leach: Ann. Rept. Mass. State Board of Health 1897, 558; 1899, 699. Food Inspection and Control (New York, 1904), p. 140.

† Identification of Pure Organic Compounds, Vol. I., p 24.

at 180°. It melts with decomposition to a red-brown liquid at 189°–192° (uncor.).

### DETERMINATION BY OXIDATION.

#### *Iodimetric Method.* *

This method depends upon the oxidation of formaldehyde to formic acid by means of iodine in alkaline solution. Two atoms of iodine oxidize one molecule of formaldehyde and the excess of iodine is liberated by acidulation and determined by titration with sodium thiosulphate.

*Reagents.* — Standard solutions of iodine and sodium thiosulphate, preferably about tenth-normal. Approximately normal solutions of sodium hydroxide and hydrochloric acid.

*Determination.* — Dilute a weighed portion of the sample with a known quantity of water so as to obtain a solution containing 0.5 to 1 per cent. of actual formaldehyde. Mix 10 c.c. of this solution with 25 c.c. normal sodium hydroxide and add from a burette 50 to 75 c.c. of tenth-normal iodine solution or enough to assure an excess of iodine as shown by the permanent yellow color of the solution. Shake or stir thoroughly and after ten minutes add 35 c.c. normal hydrochloric acid and titrate with sodium thiosulphate in the usual way using starch solution as indicator. At the same time determine the strength of the iodine in terms of the thiosulphate solution and from the amount of iodine consumed in oxidizing the formaldehyde calculate the weight of the latter in the ten c.c. taken for the determination.

*Notes.* — Under the conditions given the oxidation of formaldehyde is rapid and complete but the method is applicable only in the absence of all other substances capable of consuming iodine under these conditions. Other aldehydes, acetone and alcohol cause high results, the latter probably through absorbing iodine with the formation of chloroform.

In the absence of interfering compounds, the method is very satisfactory, even for solutions containing only one-tenth per cent. of formaldehyde. Variations in the excess of iodine added have no appreciable influence upon the results.

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* Romijn: *Ztschr. anal. Chem.*, 1897, 36, 18. Williams: Essay for the Degree of Master of Arts, Columbia University, November 1904.

### *Hydrogen Peroxide Method.**

In this method, formaldehyde is oxidized to formic acid by means of hydrogen peroxide in the presence of a known amount of alkali. The excess of alkali, over that required to combine with the formic acid produced, is determined by titration.

*Reagents.* — Standard solutions of sodium hydroxide and sulphuric acid, not weaker than normal nor stronger than twice normal. Neutral † 3 per cent. solution of hydrogen peroxide. Litmus solution as indicator.

*Determination.* — Weigh 2 to 3 grams of the solution to be tested in a stoppered flask or bottle, add 30 to 50 c.c. normal sodium hydroxide and then add cautiously from a pipette 50 c.c. of the peroxide solution, insert the stopper loosely and allow to stand at room temperature, shaking frequently until evolution of gas ceases. Working in a warm room with double normal alkali and with samples containing over 30 per cent. of formaldehyde the reaction takes place readily with considerable production of heat, and is complete within ten minutes. If normal alkali is used and the work is done in a cold room or with a more dilute solution of formaldehyde, an hour should be allowed for the oxidation of the formaldehyde by the peroxide. Finally add a few drops of litmus solution and titrate the alkali remaining uncombined. Each molecule of sodium hydroxide which has been consumed (deducting the amount required to neutralize any free acid which the peroxide solution or the original solution of formaldehyde may have contained) represents one molecule of formaldehyde oxidized to formic acid.

*Notes.* — For the best results the strength of alkali and the time allowed for the reaction should be governed by the concentration of the formaldehyde solution tested. Double normal alkali is usually recommended and the reaction is said to be complete in ten minutes with solutions containing as little as 5 per cent. of formaldehyde. For stronger solutions the method is made more accurate by using normal or sesqui-normal alkali and allowing an hour for the oxidation.

Acetaldehyde is partially oxidized under the same conditions. Its presence, therefore, causes high results but not so high as by

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* Blank and Finkenbeiner: *Ber. deut. chem. Ges.*, 1898, 31, 2979.

† If all available peroxide is acid, the acidity must be determined by titration, using litmus as indicator, and allowed for in calculating the amount of alkali consumed in the formaldehyde determination.

the iodimetric method. The results are not influenced by the presence of paraldehyde, acetone or ethyl or methyl alcohol. Commercial formalin containing only traces of acetone or acetaldehyde should show the same percentage of formaldehyde by the peroxide as by the iodimetric method.

### Determination by Condensation Reactions.

Several of the condensation reactions of formaldehyde have been utilized for its quantitative determination. One of the oldest and best-known methods is based upon the fact that formaldehyde and ammonia when mixed in not too dilute solution condense to form hexamethylene tetramine:

$$6CH_2O + 4NH_4OH = N_4(CH_2)_6 + 10H_2O.$$

If a known amount of ammonia is used, the determination of the excess shows the amount of formaldehyde originally present.

#### *Legler's Ammonia Method.**

Weigh about 1.5 grams of the solution containing 30 to 40 per cent. formaldehyde, or an equivalent amount of a more dilute solution, into a 250 c.c. glass-stoppered flask or bottle. Add 100 c.c. of fifth-normal ammonia solution; stopper tightly at once; mix and allow to stand over night at room temperature. Standing for two or three days does no harm, provided the stopper fits so tightly as to prevent any loss of ammonia. Finally, add a very small amount of rosalic acid as indicator and titrate the excess of ammonia with standard sulphuric acid. Calculate the quantity of formaldehyde originally present from the amount of ammonia consumed in condensing with it according to the equation given above.

*Notes and Precautions.* — In order to prevent loss of ammonia during the determination, the flask or bottle must be very tightly closed, the stopper being coated with vaseline if necessary. For the same reason the excess of ammonia should be titrated quickly after opening the flask. Normal or half-normal ammonia is commonly recommended for this method, but the fifth-normal solution is less likely to lose strength and has been found by Williams to give as complete reactions as the stronger solutions. In titrating

* Legler: *Ber. deut. chem. Ges.*, 1883, 16, 1333. Smith: *Journ. Amer. Chem. Soc.*, 1903, 25, 1028. Williams: *loc. cit.*

the excess of ammonia the end reaction is usually unsatisfactory, especially when the solution is highly colored by the indicator. Two drops of a freshly prepared 0.1 per cent. solution of rosalic acid has been found sufficient. With a view to obtaining a sharper end reaction, Williams recommended diluting the commercial formalin so as to obtain a solution containing about 2 per cent. of formaldehyde. Mix 10 c.c. of this solution with 40 c.c. of fifth-normal ammonia in a small glass-stoppered bottle and complete the determination as above described. The method is thus applicable to solutions containing only 1 to 2 per cent. of formaldehyde, although its results in such cases may be somewhat low.

The results are not affected by the presence of acetone, methyl or ethyl alcohol, paraldehyde or benzaldehyde. Acetaldehyde reacts with ammonia and thus causes high results if present in the formaldehyde solution.

This method, carefully carried out as described, gives results lower than those obtained by the peroxide method. The discrepancy varies greatly with different analysts and appears to be largely dependent upon the interpretation of the end-point in titrating the excess of ammonia. The color change is not sharp, but by using very little indicator a fairly definite point can be noted at which the pink tint disappears, leaving the solution nearly colorless. This is the most distinct and satisfactory end-point. It has been suggested, however, that the indistinctness of the end reaction may be due to the faintly basic character of the hexamethylenetetramine, and that the first indication of a color change should be taken as showing the point at which the excess of ammonia is neutralized. This mode of titration yields higher results which often agree with those obtained by oxidation methods. As yet there is no standard by which to judge whether the higher or the lower results are more nearly correct.

### Determination by Addition Reactions.

The general addition reaction of aldehydes with bisulphites has been used quantitatively by Ripper, as already noted. For the determination of formaldehyde, however, the reaction with potassium cyanide has been found especially useful.

#### *Potassium Cyanide Method.**

On mixing aqueous solutions of formaldehyde and potassium

* Romijn: *Ztschr. anal. Chem.*, 1897, 36, 18. Smith: *loc. cit.* Williams: *loc. cit.*

cyanide an addition product is formed, which, according to Romijn, is probably the potassium compound of oxyacetonitril:

$$CH_2O + KCN = CH_2OK \cdot CN.$$

The addition product reduces silver nitrate in alkaline solution, but has no effect in the presence of an excess of nitric acid. If, therefore, the formaldehyde to be tested be mixed with a known solution of potassium cyanide, the latter being in excess, and the mixture added to a standard solution of silver nitrate acidulated with nitric acid, only the excess of potassium cyanide reacts with the silver nitrate. The amount of formaldehyde originally present is shown by the quantity of potassium cyanide consumed in the formation of the addition product. The details of the method as here given are nearly identical with those originally recommended by Romijn.

*Reagents.*—Tenth-normal solutions of silver nitrate and ammonium thiocyanate. A solution of potassium cyanide 6.2 grams per liter. Saturated solution of ferric ammonium sulphate. Nitric acid 1.32 sp. gr. (fifty per cent.).

*Determination.*—(1) Measure 15 c.c. tenth-normal silver nitrate into a 100 c.c. flask, add 6 to 8 drops of the nitric acid and 10 c.c. of the cyanide solution; shake, dilute to the mark, mix thoroughly and filter through a dry paper. Titrate 50 c.c. of the filtrate with tenth-normal ammonium thiocyanate, using 5 c.c. of the ferric solution as indicator. The strength of the silver and of the thiocyanate solutions being known, this titration shows the strength of the cyanide.

(2) Dilute the sample until it contains about 1 per cent. of formaldehyde, mix 10 c.c. of this dilute solution with 35 c.c. of the cyanide solution and rinse the mixture into another portion of 15 c.c. tenth-normal silver nitrate, acidulated with 6 to 8 drops of the nitric acid and contained in a 100 c.c. flask; shake and determine the excess of silver by means of thiocyanate in the same way as before. Twice the difference between the two titrations (since only half the liquid was used in each case) represents the amount of cyanide consumed by the formaldehyde. If the thiocyanate solution is exactly tenth-normal, twice the difference (in c.c.) between the two titrations, multiplied by 0.003002, gives the weight of formaldehyde (in grams) in the portion taken for the determination.

*Notes.*—This method is applicable to very dilute solutions of formaldehyde, larger volumes being used in place of the 10 c.c. called for in the above directions. Smith obtained accurate results upon a solution containing 0.01 per cent.

Ethyl and methyl alcohols, acetone, benzaldehyde and paraldehyde do not interfere. Acetaldehyde causes high results if allowed to stand for some time in contact with the cyanide solution, but if the formaldehyde solution is added to the cyanide and, after mixing, poured at once into the silver nitrate solution, the presence of acetaldehyde does not influence the results. With commercially pure solutions of formaldehyde in water Romijn and Smith obtained concordant results by the iodimetric and cyanide methods. Williams obtained concordant results by the ammonia and the cyanide methods which were lower than those obtained by the oxidation methods.

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## CHAPTER V.

### Carbohydrates — General Methods.

The carbohydrates include the simple sugars (monosaccharides) and the substances which can be converted into simple sugars by hydrolysis. The monosaccharides are aldehyde alcohols or ketone alcohols, each molecule containing a carbonyl group and several hydroxyl groups, one of the latter being adjacent to the carbonyl group (Meyer-Jacobson).

The purpose of this chapter is to outline the more important analytical properties of the following carbohydrates:

Monosaccharides: Hexoses — Dextrose (d. glucose), Levulose (d. fructose), Galactose, Mannose; Pentoses — Xylose, Arabinose.

Disaccharides: Sucrose, Lactose, Maltose.

Trisaccharide: Raffinose.

Polysaccharides: Starch, Dextrin, Glycogen, Galactan, Cellulose, Pentosans.

In addition to a few notes on the occurrence, relations and solubilities of these carbohydrates, their reactions with phenylhydrazine and with alkaline copper solutions, their behavior on treatment with acids and their power of rotating the plane of polarized light will be considered.

#### OCCURRENCE AND RELATIONS.

Monosaccharides (glucoses, glycoses, monoses) have the composition $(CH_2O)_x$* and are called tetroses, pentoses, hexoses, etc., according to the number of carbon atoms in the molecule. Only pentoses and hexoses are of sufficient practical importance to call for consideration in connection with ordinary methods of analysis. The pentoses do not occur free in nature but are met by the analyst as products of the hydrolysis of the pentosans. The hexoses include all of the monosaccharides of present commercial importance and all whose biological relations have been thoroughly studied.

Dextrose (d. glucose, grape sugar, starch sugar, diabetic sugar, ordinary glucose) is widely distributed in nature, occurring especially in fruits and plant juices, often mixed with other sugars. It is a

* This statement does not apply to the methyl derivatives now frequently classified as monosaccharides.

normal constituent of blood and is the form of carbohydrate ordinarily found in the urine in diabetes or glycosuria. With the exception of the pentosans and galactan all of the di-, tri-, and polysaccharides mentioned above yield dextrose on hydrolysis.

Levulose (d. fructose, fruit sugar) occurs with dextrose in plant juices and especially in fruits and honey. It is also a product of the hydrolysis of sucrose and of raffinose.

Galactose does not occur free but, as a product of hydrolysis of lactose, raffinose and the galactans, it is of considerable analytical importance.

Mannose also is not found free but has been detected among the products of hydrolysis of the insoluble carbohydrate matter of a number of thick-walled vegetable tissues, nut shells, etc., and of several Japanese vegetables.

The disaccharides considered here are all hexo-bioses ($C_{12}H_{22}O_{11}$).

Sucrose (saccharose, cane sugar) is widely distributed in the vegetable kingdom being found in considerable quantity, generally mixed with dextrose and levulose, in the fruits and juices of many plants. The most important sources of sucrose are the sugar beet, the sugar and sorghum canes, and the sugar maple. A molecule of sucrose yields on hydrolysis one molecule each of dextrose and levulose. The hydrolysis of sucrose is often called "inversion" and the resulting mixture of equal parts dextrose and levulose is known as "invert-sugar."

Lactose (lactobiose, milk sugar) occurs in the milk of most mammals, constituting usually from 4 to 7 per cent. of the fresh secretion. Lactose crystallizes with one molecule of water which it retains on drying at room temperature over sulphuric acid or on heating in the air at 100°, but loses at about 130°. A molecule of lactose yields on hydrolysis one molecule each of dextrose and galactose.

Maltose (malt sugar) is formed from starch by the action of diastatic enzymes and is therefore an important constituent of germinating cereals, malt, malt extract and beer wort. It is also formed as an intermediate product when starch is hydrolyzed to dextrose by boiling with dilute mineral acids, as in the manufacture of commercial glucose. Maltose crystallizes with one molecule of water which it loses on heating in the air at 100°. Each molecule of maltose yields two molecules of dextrose on hydrolysis.

The only trisaccharide of practical importance is raffinose

($C_{18}H_{32}O_{16}$) also called *meletriose* and formerly *melitose* or *gossypose*. It occurs in cotton seed and in small quantity in the germs of various other seeds including wheat and barley. Sugar beets, especially if unhealthy or injured, sometimes contain raffinose in sufficient quantity to affect the refining process. Raffinose crystallizes with five molecules of water in needles or slender prisms and has a marked influence upon the crystallization of the cane sugar present.* Raffinose loses its water of crystallization at 100°. On hydrolysis it yields one molecule each of dextose, levulose and galactose. Partial hydrolysis results in the formation of levulose and the disaccharide, *melibiose*.

Starch $(C_6H_{10}O_5)_x$ is the most important of the polysaccharides being the principal form of carbohydrate in grains and most other edible seeds, as well as in potatoes and other tubers. It is the main product of the assimilation process and the principal reserve carbohydrate of most green plants. Commercially it is of great importance as a constituent of foods, as the source of dextrin, maltose and commercial glucose and as the principal raw material of many of the fermentation industries. Starch constitutes over one-half of the solid matter of all ordinary cereals and about three-fourths of the total solids in potatoes. Starch granules of different plants vary in size and structure so that in most cases the source of a starch which has not been altered by heat, ferments, or chemical reagents, can be determined by microscopical examination. All starches yield dextose only, as the final product of complete hydrolysis.

Dextrins, $(C_6H_{10}O_5)_x$ or $(C_6H_{10}O_5)_x \cdot H_2O$, are formed from starch by the action of enzymes, acids or heat. Small amounts of dextrin are found in normal, and larger amounts in germinating, cereals. Malt diastase acting upon starch in fairly concentrated solution yields usually about one part of dextrin to four of maltose. During acid hydrolysis, dextrin is formed as an intermediate product between soluble starch and maltose. Commercial dextrin, the principal constituent of "British gum," is obtained by heating starch, either alone or with a small amount of dilute acid.

Glycogen, $(C_6H_{10}O_5)_x$ or perhaps $(C_6H_{10}O_5)_x \cdot H_2O$, is the principal carbohydrate of the animal organism being found in small quantity in the muscles and more abundantly in the liver of all well-nourished animals. It is a white amorphous powder intermediate

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* Stone and Baird: *Journ. Amer. Chem. Soc.*, 1897, 19, 116.

in properties between starch and dextrin and is sometimes called animal starch. The determination of glycogen is often important in physiological investigations and is sometimes useful in distinguishing horseflesh from beef, the latter containing usually less than 0.7 per cent. of glycogen, the former often two or three times this amount. On complete hydrolysis glycogen yields only dextrose.

Galactans, amorphous polysaccharides yielding galactose on hydrolysis, occur in small quantity in many plants and in relatively large amount in the seeds of the legumes where they largely replace starch as reserve carbohydrate. Since the galactans are readily hydrolyzed by hot dilute acids and are digested by some of the diastatic enzymes, it is probable that galactan has been reported as starch in many analyses.

Cellulose occurs in the cell walls of all vegetable tissues. The term is sometimes applied to the whole of the fiber which is unattacked by boiling dilute acids and alkalies but should be restricted to that constituent of the fiber which is of a true carbohydrate nature. "Normal" cellulose, such as is derived from cotton and flax fibers, yields dextrose on hydrolysis. A few celluloses have been found to yield mannose or a pentose (probably xylose) in addition to dextrose (Tollens).

Pentosans, anhydrides of arabinose and xylose, are the principal constituents of the vegetable gums, araban occurring especially in the soluble gums such as cherry gum and gum arabic, xylan in the so-called woodgum of fibrous tissues such as wood, straw, vegetables, and the outer portion of the cereal grains. The wheat grain, for example, contains 3 to 5 per cent. of pentosan which in the milling process is largely left in the bran. The so-called patent flour obtained from the interior of the grain contains hardly any pentosan while the breakfast cereals and the so-called entire wheat and graham flours have usually about as much as the original grain.

## SOLUBILITIES.

### In Water.

Of the carbohydrates mentioned above, all except the polysaccharides are crystallizable compounds dissolving in water to form clear solutions. Milk sugar dissolves in six parts of water at ordinary temperature; all of the other members are more freely solu-

ble. Among the polysaccharides, dextrin, glycogen and some of the galactans and pentosans are soluble; starch, cellulose, some of the galactans and most of the pentosans of ordinary food materials are insoluble in cold water. Glycogen gives a strongly opalescent solution, which is not cleared by repeated filtration but loses its opalesence on the addition of a little potassium hydroxide or acetic acid. On heating with water, starch grains swell and finally gelatinize with the formation of "starch paste." Different starches vary considerably in the temperature at which they gelatinize and in the physical properties of the paste produced. Thin starch pastes can be filtered through paper, but almost always leave some gelatinous residue upon the filter. Pastes containing only a few hundredths of one per cent of starch become clear on boiling and can be filtered without loss. Water-soluble starch can be prepared* by chemical treatment and is sometimes found in natural products, for example in immature grains.

Cellulose, and the ordinary pentosans of foods and fibers, are insoluble in water and not gelatinized by boiling.

### In Alcohol and Ether.

Levulose is soluble in 5 parts of cold absolute alcohol and is somewhat soluble in mixtures of ether and strong alcohol. The other monosaccharides are sparingly soluble in cold alcohol, insoluble in ether and practically insoluble in the alcohol-ether mixture. Dextrose is much more readily soluble in hot than in cold alcohol; 100 parts of 90 per cent. alcohol dissolve about 2 parts dextrose at 18°, about 22 parts at boiling temperature.

The di-, tri-, and polysaccharides are insoluble in ether. Di- and trisaccharides are less soluble in alcohol than is dextrose. Lactose is practically insoluble in alcohol, even when the latter is diluted to 60 per cent.

Sucrose is much more readily soluble in diluted than in concentrated alcohol. According to Scheibler:

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 | parts | 90 | per cent. | alcohol | dissolve | 0.9 | parts | at | 14°; | 2.3 | parts | at | 40°. |
| " | " | 80 | " | " | " | 6.6 | " | " | " | 13.3 | " | " | " |
| " | " | 70 | " | " | " | 18.8 | " | " | " | 31.4 | " | " | " |
| " | " | 60 | " | " | " | 33.9 | " | " | " | 49.9 | " | " | " |
| " | " | 50 | " | " | " | 47.1 | " | " | " | 63.4 | " | " | " |

* Lintner: *Journ. prakt. Chem.* 1886, [2] 34, 381. Wroblewski: *Ztschr. physiol. Chem.*, 1898, 24, 173. Vaubel: Best. org. Verbind., II., 500.

Sucrose dissolves in about 80 parts of boiling absolute alcohol. All of the polysaccharides are insoluble in alcohol. Those which are soluble in water can be precipitated from their aqueous solutions by the addition of strong alcohol.

### In Acids and Alkalies.

Among the carbohydrates which are inslouble in water, separations can sometimes be made by the use of acid or alkaline solutions.

Cellulose is soluble in concentrated sulphuric acid, but insoluble in any ordinary aqueous solution of acid or alkali. It dissolves in Schweitzer's reagent (aqueous ammonia saturated with cupric hydroxide) to a viscous solution from which it is precipitated by neutralization with acid.

Starch is insoluble in Schweitzer's reagent or in solutions of ammonia. Treated with dilute aqueous solutions of sodium or potassium hydroxide, starch swells, gelatinizes and becomes soluble. It can be completely precipitated from such a solution by neutralizing with acetic acid and adding strong alcohol. Starch is not affected by dilute solutions of alkalies in strong alcohol. By diluted solutions of strong acids starch is first dissolved, then hydrolized. Some weak organic acids dissolve starch with little if any hydrolysis. Boiling water containing 1 per cent. of salicylic acid dissolves starch to an opalescent solution which filters much more readily than a corresponding starch paste made with water alone.

The pentosans of foods and fibers — so-called woodgums consisting mainly of xylan — are insoluble in dilute ammonia or in Schweitzer's reagent, largely soluble in dilute aqueous solutions of sodium or potassium hydroxide and in cold dilute acids. From such solutions the pentosan is precipitated by alcohol. Boiling dilute mineral acids dissolve and hydrolize pentosans almost as readily as starch. Hence pentosans have frequently been reported as starch when the latter has been estimated by direct hydrolysis with acid and determination of the resulting glucose.

### REACTIONS WITH PHENYLHYDRAZINE.

All of the monosaccharides, and maltose and lactose among the disaccharides, contain the free carbonyl radical and, therefore, on treatment with phenylhydrazine acetate in molecular proportions, they react to form hydrazones. All of these hydrazones, except

that of mannose, are freely soluble in water. The formation of an insoluble hydrazone is a distinctive test for mannose, and has been applied as a quantitative method by Bourquelot and Herissey.*

When the hydrazone of a sugar is heated with an excess of phenylhydrazine, the hydroxyl adjacent to the hydrazone group undergoes oxidation and condensation resulting in the formation of the dihydrazone, more commonly known as the osazone. The osazones are only sparingly soluble in water, and on crystallization show definite forms and melting points. The formation of the osazone is, therefore, an important means of identification for sugars having the carbonyl radical.

PREPARATION AND PROPERTIES OF THE OSAZONES.

*To form the osazone,*† dissolve one part of the sugar, two of pure phenylhydrazine hydrochloride and three of crystallized sodium acetate in 20 parts of water in a test-tube. Cork loosely to avoid evaporation and place the tube in boiling water.

If the substance tested is pure and the above proportions are maintained, the time required for the appearance of an osazone precipitate is fairly constant. These differences in the rate of osazone formation have been especially studied by Mulliken and play a prominent part in his system of identification of pure sugars. All of the monosaccharides, and no other carbohydrates, give precipitates within 20 minutes. Maltose and lactose form osazones which are much more soluble in hot water than those of the monosaccharides, and under the conditions given, separate from the solution only on cooling. Sucrose when subjected to this test is gradually hydrolyzed so that eventually it yields the osazone of dextrose and levulose. Under the conditions given, however, this requires about 30 minutes heating in the water bath.

In general, therefore, a precipitate appearing at the temperature of the water bath within 20 minutes indicates the presence of a monosaccharide; a slight precipitate of osazone appearing only on cooling might be due to a very small amount of monosaccharide, but any considerable quantity of osazone which precipitates

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* *Journ. Pharm. Chim.*, 1899, [6], 10, 206. See also Pellet: *Bull. Assoc. Chim. Sucr. Distill.*, 1900–01, 18, 758; Abs. *Ztschr. Unters. Nahr. Genussm.*, 1902, 5, 74.

† Fischer: *Ber. deut. chem. Ges.*, 1884, 17, 579; 1887, 20, 821; 1888, 21, 1805, 2631; 1889, 22, 87; 1890, 23, 2117.

Mulliken: Identification of Pure Organic Compounds, Vol. I., p. 32.

only on cooling is indicative of lactose, maltose or isomaltose.

The maximum yield of osazone is usually obtained by warming the solution in the water-bath for one to two hours and then allowing it to cool. The osazone thus obtained is ordinarily a yellow iridescent precipitate, more or less distinctly crystalline according to the purity and concentration of the solution. To purify it, filter on a small paper, wash with a little cold water, dissolve in the smallest possible amount of boiling 50 per cent. alcohol and filter hot. The osazone which separates from the alcohol solution may, if desired, be further recrystallized from alcohol or from pyridine.

Dextose, levulose and mannose yield the same osazone, *glucosazone*, which crystallizes in needles melting at 204°-205° when heated at such a rate that the melting-point is reached in three to four minutes.* *Galactosazone* crystallizes in needles which melt at 193°; *maltosazone*, in independent needles or tables melting at 206°; *lactosazone*, in masses of microscopic prisms melting at 200°. *Xylosazone* and *arabinosazone* melt at about 160°.

A conclusive method of ascertaining whether an osazone is that of a pentose, a hexose or a disaccharide is to determine the percentage of nitrogen. In the case of a mixture, the hexosazone can be freed from osazones of the other two groups by washing with hot water. Maltosazone is soluble in about 75 parts, xylosazone in about 50 parts, and lactosazone in 80 to 90 parts of boiling water, while the pure hexosazones are nearly insoluble.

*The yield of osazone* has been studied especially by Macquenne,† Laves,‡ Fischer,§ Lintner and Kröber,|| Raimann,¶ and Davis and Ling.** In each of these cases the osazone was formed by heating for one or two hours, in a more dilute solution than that above given, with a considerable excess of a slightly acid solution of phenylhydrazine acetate. The precipitate of osazone can then be filtered, washed with water and weighed. According to Laves

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* Any of the osazones on being heated very slowly may begin to melt considerably below the temperatures given.

† *Compt. rend.*, 1891, 112, 799; *Ztschr. anal. Chem.*, 1894, 33, 226.

‡ *Archiv. der Pharm.*, 1893, 231, 366; Vaubel's Bestimmung organischer Verbindungen, II., 307, 311, 312.

§ *Ber. deut. chem. Ges.*, 1895, 28, 1437.

|| *Ztschr. f. d. ges. Brauwesen*, 1895, 18, 153; *Ztschr. anal. Chem.*, 1896, 35, 95; *Analyst*, 1897, 20, 167.

¶ *Ztschr. anal. Chem.*, 1901, 40, 390.

** *Journ. Chem. Soc.*, 1904, 85, 24.

the following corrections should be applied for the amount of glucosazone left in solution or dissolved by washing:

| | |
|---|---|
| 100 parts boiling water dissolve | 0.01 part osazone. |
| 100 " water at 20° " | 0.0042 " " |
| 100 " 2 per cent. acetic acid at 20° dissolve | 0.007 " " |
| 100 " 3 " " " " " 20° " | 0.0145 " " |
| 100 " 4 " " " " " 20° " | 0.022 " " |
| 100 " 5 " " " " " 20° " | 0.031 " " |
| 100 " 10 " " alcohol slightly acidulated dissolve at 20° | 0.0075 " " |

Corresponding corrections have not been worked out for other osazones, as the attempts to apply the osazone method quantitatively have been mainly with the object of determining dextrose or dextrose and levulose. In comparative tests carried out in exactly the same manner, the weight of osazone obtained is proportional to the amount of sugar originally present, but slight differences of manipulation affect the yield to such an extent that the osazone precipitation cannot yet be regarded as a satisfactory quantitative method.

*For the detection of dextrose*, or invert sugar, in the absence of other monosaccharides, the osazone test may be applied as follows:* Dissolve 0.2 gram of sample (or, if only partially soluble, stir so much as contains 0.2 gram of soluble solids) in 4 c.c. of water, filter if necessary, add 0.40 gram phenylhydrazine hydrochloride and 0.60 gram crystallized sodium acetate; shake until dissolved, filter if not clear; place the solution, contained in a small test-tube corked loosely to prevent evaporation, in a beaker of boiling water and shake frequently without removing the tube from the water-bath.

The following results were obtained with solutions containing known amounts of pure sugars, the amounts of water and reagents and the manipulation being in each case as just described.

SOLUTIONS OF PURE DEXTROSE.

| | | | | | | |
|---|---|---|---|---|---|---|
| Dextrose present | 0.10 | 0.05 | 0.02 | 0.01 | 0.005 | gram. |
| Osazone appeared in | 5 | 6½ | 10½ | 17–19 | 34 | minutes. |

SOLUTIONS CONTAINING 0.01 GRAM DEXTROSE, WITH VARYING AMOUNTS OF MALTOSE OR LACTOSE.

| | | | | | |
|---|---|---|---|---|---|
| Maltose or Lactose present | 0.01 | 0.05 | 0.10 | 0.20 | gram |
| Osazone appeared in | 22–25 | 30–32 | 37–46 | † | minutes. |

* Compare Mulliken, *loc. cit.*

† No precipitate of osazone appeared in the hot solution although the heating was continued for two hours.

These experiments, made by R. H. Williams in the course of an investigation which is still in progress in this laboratory, illustrate the delicacy of the test when only dextrose is present, and the influence of maltose and lactose in retarding the precipitation of dextrose as osazone. Sucrose up to ten times the weight of dextrose present had no appreciable influence. In larger proportion it may hasten very slightly the appearance of the osazone. As already explained sucrose tested alone undergoes gradual hydrolysis and thus finally gives a precipitate of glucosazone.

The delicacy of the test in the presence of dextrin (which is known to retard the formation of the osazone), and of substances other than carbohydrates, has not yet been studied in detail, except in the case of certain constituents of urine.* Hence in applying the osazone test to an unknown solution, it is well to make at the same time two check experiments, one with a mixture of carbohydrates corresponding to that which the unknown solution is believed to contain, the other with a portion of the unknown solution to which has been added a very small amount of dextrose.

## REDUCTION OF ALKALINE COPPER SOLUTIONS.

The aldehyde and ketone sugars are easily oxidized and hence have the power of reducing certain metallic salts, especially salts of copper, silver and mercury in alkaline solution. The reagent most commonly used in detecting or determining sugars by their "reducing power," is prepared by treating cupric sulphate with an excess of an alkaline tartrate solution. When such an alkaline copper solution is boiled with any one of the aldehyde or ketone sugars (*i. e.*, with any carbohydrate capable of reacting with phenylhydrazine), the copper is reduced by the action of the sugar from the cupric to the cuprous state. In the case of a tartrate solution containing only fixed alkali, the reduced copper is precipitated as cuprous oxide. The changes which take place in the sugar are quite complex and only in the case of dextrose have the products been studied to any extent.

On boiling with the alkaline copper solution, dextrose is attacked in two ways:† (1) *Oxidation* to tartronic, gluconic, oxalic, acetic,

* The influence of other substances likely to be present, upon the osazone test for dextrose in urine has been studied by — Hirschl; *Ztschr. physiol. Chem.*, 1890, **14**, 377: Jaffé; *Ibid.*, **22**, 532: Neuberg; *Ibid.*, 1900, **29**, 274: Neuman-Wender; *Pharmac. Post.*, **26**, 573, 614 (Vaubel; *l. c.* II., 309).

† For fuller discussions of this subject see the works of Lippmann, Vaubel and Wiley cited below.

formic and carbonic acids, and probably other products which have not yet been recognized. (2) *Decomposition by alkali* into substances no more highly oxidized than the original sugar. Lactic acid is the most conspicuous of these, but other more complex products, including aromatic compounds and gummy substances of unknown constitution, are formed by rearrangement under the influence of the alkali. In practice less than half as much copper is reduced as would correspond to a complete oxidation of dextrose to tartronic acid, although the latter compound is probably the chief oxidation product formed. It is evident, therefore, that the decomposing action of the alkali, unless carefully controlled, will influence greatly the amount of copper reduced.

Fehling,* who first systematically studied the determination of reducing sugars by their action upon alkaline copper solutions, used a reagent containing 34.64 grams of crystallized copper sulphate, 138.6 grams of potassium tartrate and 47 to 55 grams of sodium hydroxide, the whole dissolved in water and diluted to one liter. Ten c.c. of this reagent were diluted with 40 c.c. of water and heated to boiling. A solution containing not more than 1 per cent. of dextrose or invert-sugar was then added, until after two minutes boiling the solution contained no unreduced copper. Fehling held that under these conditions each molecule of dextrose or levulose reduced five atoms of copper from the cupric to the cuprous state. According to this proportion 10 c.c. of the Fehling reagent require for complete reduction exactly 0.05 grams of dextrose, levulose or invert sugar.

It has been found, however, that the ratio between dextrose and copper is constant only when the alkalinity and dilution of the solution are kept nearly uniform. According to Soxhlet,† one molecule of dextrose in 1 per cent. solution reduces 5.26 atoms of copper from undiluted Fehling solution or 5.055 atoms from the same solution diluted with four times its volume of water. Soxhlet recommended the use of 1 per cent. solutions of reducing sugars with undiluted Fehling solution under which conditions 10 c.c. of the latter are reduced by 0.0475 gram of dextrose. The use of undiluted Fehling solution has, however, the disadvantage that sucrose, if present, may be partially hydrolyzed by the boiling alkali thus causing high results, whereas the diluted Fehling solu-

* *Ann. Chem.*, 1849, 72, 106.

† *Journ. prakt. Chem.*, 1880 [2], 21, 227.

tion seems to be entirely without effect upon sucrose. Moreover, it is evident from the figures given by Soxhlet that at a slightly greater dilution, as when a solution containing 0.25 to 0.5 per cent. of dextrose is used, the ratio of Fehling is practically correct. Soxhlet also showed that the reducing power is constant only when the whole amount of sugar required for the reduction of the copper is added at once.

Many other modifications of Fehling's method have been proposed and a committee of the International Commission for Unifying Methods of Sugar Analysis is now (1905) investigating this subject with a view to the development of more satisfactory and uniform methods. Until some such international agreement is reached the volumetric determination of reducing sugars may conveniently be made by the following method, which is that of Fehling with only such modifications as have been generally recognized as essential to accuracy.

### Fehling's Volumetric Method.

*Reagents.*—(1) Copper solution: Dissolve 34.64 grams of pure crystallized copper sulphate in water, and dilute to exactly 500 c.c.

(2) Alkaline tartrate solution: Dissolve 175 grams of pure sodium potassium tartrate and 50 grams of pure sodium hydroxide in water, and dilute to 500 c.c. This reagent does not keep well unless carefully protected from the air.

*Determination.*—Measure accurately into a small flask or casserole or a deep porcelain dish, 5 c.c. of each of the above solutions, making 10 c.c. of the "mixed Fehling reagent." Add 40 c.c. of water, mix and boil. To the boiling liquid, add from a burette a solution which is judged to contain about 1 per cent. of the sugar to be determined, until after two minutes boiling the blue color is entirely discharged showing that all of the copper has been reduced. This test indicates approximately the amount of reducing sugar in the sample. Now adjust the strength of the sugar solution, if necessary, so that between 10 and 20 c.c. will be required to reduce 10 c.c. of the mixed Fehling reagent. Repeat the test, adding the calculated amount of sugar solution at once to the boiling copper solution; regulate the heating so that the mixture will begin to boil about one minute after the addition of the sugar; note the exact time that actual boiling commences and continue to boil for just two minutes, then remove the flame and at once test the liquid for un-

reduced copper. This may be done in several ways. The method commonly recommended is to filter a portion of the liquid, acidulate with acetic acid and test for copper with potassium ferrocyanide. A more convenient method which appears to be equally reliable has recently been suggested by Harrison * and consists in adding a drop or two of the liquid (which need not be filtered from cuprous oxide) to a considerable excess of cold acidulated starch-iodide solution prepared as described below. Any unreduced copper causes a liberation of iodine with the production of a color varying from purplish red to deep blue according to the amount of copper in the solution. Until considerable experience has been acquired, it is well to make both tests, observing all the precautions noted below.

Repeat the test using more or less of the sugar solution depending upon the presence or absence of an excess of copper in the preceding experiment until two amounts of sugar solution are found which differ by only 0.1 or 0.2 c.c., one giving complete reduction and the other leaving a small amount of copper in the solution. The mean of the two readings is taken as the volume of solution required for complete reduction of the copper reagent.

*Notes and Precautions.* — All of the reagents used must be the purest obtainable and the two constituents of the "mixed Fehling reagent" must be kept separate instead of being made up in one solution as was formerly done. If the solutions are not fresh, make a blank test in a casserole, boiling as above without the addition of any sugar, allow to stand for a few minutes, then decant off the liquid and notice whether any cuprous oxide has been precipitated. Finally wipe out the casserole with a small piece of filter paper and examine the latter, which may show traces of cuprous oxide not visible in the presence of the blue solution. If the liquid shows any change of color in this blank test, or if the slightest trace of cuprous oxide is found, the alkaline tartrate solution must be rejected and another blank test made with a freshly prepared solution.

If the sample analyzed is rich in reducing sugar all of the quantities given above should be doubled in order to reduce the influence of unavoidable errors.

In testing for copper by the ferrocyanide method, great care must be taken to filter out all of the cuprous oxide quickly. This is best done by withdrawing the portion to be tested through the

* *Pharm. Journ.*, 1903, 170; Sutton's Volumetric Analysis (9th Ed.), p. 312.

filtering tube described by Wiley * or by pouring it carefully upon a double or triple paper filter. A single filtration through one ordinary paper seldom removes all of the cuprous oxide. On the other hand if a slight trace of unreduced copper is present this may sometimes be lost by repeatedly passing the solution through several thicknesses of paper. Traces of iron getting into the solution from reagents or apparatus may cause a slight coloration with ferrocyanide. Only a distinct red-brown precipitate or coloration should be taken as showing the presence of unreduced copper.

The starch-iodide solution for detecting unreduced copper without filtration is prepared as follows: Boil 0.02 gram starch with 15 to 20 c.c. of water, cool, add 4 to 5 grams potassium iodide and dilute to 50 c.c. A fresh solution must be prepared each day. When the test is to be made pour about 1 c.c. of the starch-iodide into a test-tube, add two or three drops of acetic acid and then immediately a drop or two of the solution to be tested.

*Calculation and Verification of Results.*— In calculating the results it is commonly assumed that 10 c.c. of the mixed Fehling reagent require for reduction under the above conditions:

0.0500 gram of anhydrous dextose, levulose or invert sugar.
0.0678 " " dry crystallized lactose ($C_{12}H_{22}O_{11} \cdot H_2O$).
0.0807 " " anhydrous maltose.

That these factors are not absolute, but are dependent upon exact uniformity of conditions has already been explained. To secure the greatest accuracy, therefore, the result obtained should be verified by a check experiment, carried out under the exact conditions of the analysis, with a known solution of pure sugar of the kind actually determined.

According to Soxhlet, levulose and galactose have distinctly less reducing power than dextrose. It is quite commonly assumed, however, that these three monosaccharides have the same reducing power, but that invert sugar often fails to show its full effect because of the decomposing action of the acid used for inversion. Xylose and arabinose reduce Fehling solution somewhat more strongly than does dextrose.

## *Substitution of Other Methods.*

Aside from modifications in detail, several methods intended to replace that of Fehling have been proposed. In some of these the

* Agricultural Analysis, Vol. III., p. 130.

caustic alkali of Fehling's solution is replaced by a weaker base, such as alkaline carbonate or bicarbonate (Soldani, Sidersky, Ost) or ammonia (Allein and Gaud); in others, ammonia (Pavy) or cyanide (Gerrard) is added to the usual Fehling reagent in order to hold in solution the reduced copper, so that the point of complete reduction can be directly observed by the disappearance of the blue color. More important, however, are the methods in which, as described below, a fixed volume of the sugar solution is boiled with an excess of the alkaline copper solution and the reduced copper collected and determined.

### DEFREN'S GRAVIMETRIC METHOD.*

This method is based on that of O'Sullivan † and provides a uniform procedure for the determination of dextrose, maltose or lactose.

*Reagents.*—(1) Dissolve 34.64 grams of copper sulphate in water, add 0.5 c.c. strong sulphuric acid and dilute to 500 c.c.

(2) Dissolve 178 grams of sodium potassium tartrate and 50 grams of sodium hydroxide in water and dilute to 500 c.c.

*Determination.*—Mix 15 c.c. of each of the above reagents in an Erlenmeyer flask having a capacity of 250 to 300 c.c., dilute with 50 c.c. of freshly boiled distilled water and place the flask in a boiling water-bath for five minutes; then add 25 c.c., accurately measured from a burette or pipette, of a solution containing approximately 0.5 per cent. of the sugar to be determined and allow the mixture to stand in the boiling water-bath for fifteen minutes. Remove the flask from the bath and filter as quickly as possible (using moderate suction) through asbestos prepared as described below; wash the cuprous oxide with boiling distilled water until the filtrate is no longer alkaline. The cuprous oxide can now be (1) washed with alcohol and then with ether, dried in a boiling water oven for 20 minutes and weighed; (2) ignited and weighed as cupric oxide, as recommended by Defren; or (3) dissolved in nitric acid and the copper determined by electrolysis or by any other reliable method in which case it will not be necessary to use asbestos especially prepared by boiling with acid and alkali. From the weight of copper or cuprous oxide determined, calculate the equivalent amount of cupric oxide and find the corresponding weight of reducing sugar from Defren's table.

* *Journ. Amer. Chem. Soc.*, 1896, 18, 749.

*Precautions.* — If the copper reduced is to be weighed as cuprous or cupric oxide on the asbestos filter, the latter must be especially prepared in order that it shall lose no weight when treated with the hot alkaline Fehling solution. Asbestos of good quality is boiled with nitric acid (1.05 to 1.10 sp. gr.) washed with water then boiled with 25 per cent. sodium hydroxide, washed, and the treatment with acid and alkali repeated. The prepared asbestos is used to make a tight felt about one centimeter thick in a Gooch crucible. When the crucible has been prepared for use and weighed it should be tested by running through it a "blank" of hot alkaline Fehling solution and washing with water as in a regular determination. The loss of weight should not exceed one half milligram. After each determination the precipitate is dissolved in nitric acid and the crucible washed, ignited and reweighed. If a loss of over one milligram is found, the determination should be rejected and the filter treated alternately with acid and alkali until it ceases to lose in weight.

*Notes on Defren's Table.* — Defren determined the amount of copper reduced by fifteen to twenty known solutions each of dextrose, maltose and lactose with the following results:

$$\text{Dextrose} = (0.4400 + 0.000037\,W)W.$$
$$\text{Maltose} = (0.7215 + 0.000061\,W)W.$$
$$\text{Lactose} = (0.6270 + 0.000053\,W)W.$$

In which $W$ is the weight of cupric oxide obtained, the values of $W$ varying from 30 to 320 milligrams.

From these formulæ it is apparent that the reducing power of each of the sugars increases slightly with the concentration, as was found to be the case in Soxhlet's volumetric method.

The table on the following page is based on these equations. This table is condensed from that given in Defren's paper (*l. c.*) in which the weights of sugars corresponding to each milligram of cupric oxide are stated. In using either table the weight of copper or cupric oxide should be taken to one-tenth milligram and the corresponding weight of reducing sugar found by the method of proportional parts.

*Defren's Table for Dextrose, Maltose and Lactose.*

| Cupric Oxide mgms. | Dextrose mgms. | Maltose mgms. | Lactose mgms. | Cupric Oxide mgms. | Dextrose mgms | Maltose mgms. | Lactose mgms. |
|---|---|---|---|---|---|---|---|
| 30 | 13.2 | 21.7 | 18.8 | 180 | 80.4 | 131.8 | 114.6 |
| 40 | 17.6 | 29.0 | 25.2 | 190 | 84.9 | 139.1 | 121.0 |
| 50 | 22.1 | 36.2 | 31.5 | 200 | 89.5 | 146.6 | 127.5 |
| 60 | 26.5 | 43.5 | 37.8 | 210 | 94.0 | 154.1 | 134.1 |
| 70 | 30.9 | 50.8 | 44.1 | 220 | 98.6 | 161.5 | 140.6 |
| 80 | 35.4 | 58.1 | 50.5 | 230 | 103.1 | 169.1 | 147.0 |
| 90 | 39.9 | 65.5 | 56.8 | 240 | 107.7 | 176.6 | 153.5 |
| 100 | 44.4 | 72.8 | 63.2 | 250 | 112.3 | 184.1 | 160.0 |
| 110 | 48.9 | 80.1 | 69.5 | 260 | 116.9 | 191.6 | 166.5 |
| 120 | 53.3 | 87.4 | 75.9 | 270 | 121.4 | 199.2 | 173.0 |
| 130 | 57.8 | 94.8 | 82.4 | 280 | 126.1 | 206.8 | 179.6 |
| 140 | 62.2 | 102.1 | 88.7 | 290 | 130.7 | 214.3 | 186.2 |
| 150 | 66.8 | 109.5 | 95.2 | 300 | 135.3 | 221.9 | 192.8 |
| 160 | 71.3 | 116.9 | 101.7 | 310 | 139.9 | 229.6 | 199.3 |
| 170 | 75.8 | 124.4 | 108.2 | 320 | 144.5 | 237.2 | 205.9 |

## KJELDAHL'S GRAVIMETRIC METHOD.*

As the result of study of many of the conditions affecting the gravimetric determination of reducing sugars, Kjeldahl recommended the following method:

*Reagents.*—(1) Copper Solution: 34.65 grams of pure crystallized copper sulphate in 500 c.c. (2) Alkaline Solution: 65 grams of sodium hydroxide, determined by titration, in 500 c.c. (3) Pure sodium potassium tartrate pulverized and kept in the dry state.

*Determination.*—In an Erlenmeyer flask holding about 150 c.c., mix 5.2 grams of sodium potassium tartrate and 15 c.c. each of the alkaline and copper solutions,† add a known volume of the sugar solution, which must not contain over 0.140 gram of dextrose or an equivalent amount of other reducing sugar, and fill with water to 100 c.c.; close the flask with a two-holed stopper and pass a current of hydrogen or illuminating gas until all air is expelled from the flask; stand the flask in a boiling water bath for exactly 20 minutes; filter, and determine the reduced copper as described under Defren's method. Find the corresponding weight of reducing sugar from the table on the opposite page.

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* *Midelelser fra Carlsberg Lab.*, 1895, **5**, 1; Abs. *Analyst*, 1895, **20**, 227; *Ztschr. anal. Chem.* 1896, **35**, 344; Bujard and Baier's Hilfsbuch für Nahrungsmittelchemiker, Berlin, 1900.

† This gives practically 30 c.c. of the "mixed Fehling reagent" containing the same quantity of copper as is used in Defren's method. Kjeldahl's method also includes directions and tables for the use of 15 c.c. or 50 c.c. of the mixed copper reagent if necessary. The proportions here given are adapted to such solutions as would usually be obtained in the estimation of reducing sugars.

*Kjeldahl's Table for Reducing Sugars.*

| Copper mgms. | Dextrose mgms. | Levulose mgms. | Invert-Sugar mgms. | Galactose mgms. | Lactose $C_{12}H_{22}O_{11}+H_2O$ mgms. | Maltose $C_{12}H_{22}O_{11}$ mgms. |
|---|---|---|---|---|---|---|
| 40 | 17.8 | 19.8 | 19.1 | 19.8 | 26.7 | 30.9 |
| 50 | 22.4 | 24.9 | 23.9 | 25.0 | 33.6 | 38.8 |
| 60 | 27.0 | 30.1 | 28.8 | 30.1 | 40.6 | 46.8 |
| 70 | 31.8 | 35.2 | 33.8 | 35.4 | 47.6 | 54.8 |
| 80 | 36.6 | 40.5 | 38.8 | 40.7 | 54.6 | 63.0 |
| 90 | 41.4 | 45.9 | 43.9 | 46.0 | 61.8 | 71.2 |
| 100 | 46.4 | 51.3 | 49.1 | 51.5 | 69.0 | 79.4 |
| 110 | 51.4 | 56.8 | 54.4 | 57.0 | 76.4 | 87.7 |
| 120 | 56.6 | 62.4 | 59.8 | 62.6 | 83.8 | 96.1 |
| 130 | 61.8 | 68.0 | 65.2 | 68.3 | 91.3 | 104.7 |
| 140 | 67.1 | 73.7 | 70.7 | 74.0 | 98.9 | 113.3 |
| 150 | 72.5 | 79.6 | 76.3 | 79.9 | 106.5 | 121.9 |
| 160 | 78.0 | 85.5 | 82.0 | 85.8 | 114.3 | 130.7 |
| 170 | 83.7 | 91.5 | 87.9 | 91.9 | 122.2 | 139.6 |
| 180 | 89.4 | 97.6 | 93.8 | 98.0 | 130.3 | 148.6 |
| 190 | 95.3 | 103.9 | 99.9 | 104.3 | 138.4 | 157.6 |
| 200 | 101.4 | 110.2 | 106.1 | 110.6 | 146.7 | 166.8 |
| 210 | 107.5 | 116.7 | 112.4 | 117.1 | 155.0 | 176.1 |
| 220 | 113.9 | 123.2 | 118.8 | 123.8 | 163.5 | 185.5 |
| 230 | 120.4 | 130.0 | 125.5 | 130.5 | 172.2 | 195.1 |
| 240 | 127.1 | 136.8 | 132.2 | 137.5 | 181.0 | 204.7 |
| 250 | 134.1 | 143.8 | 139.2 | 144.5 | 189.9 | 214.4 |
| 260 | 141.2 | 150.9 | 146.3 | 151.8 | 199.0 | 224.3 |

BARFOED'S CUPRIC ACETATE METHOD.*

For the detection of dextrose Barfoed used a slightly acid solution of cupric acetate in place of the Fehling solution. Lieben attempted to determine dextrose quantitatively in the presence of maltose by means of such solutions. This test, like the formation of the osazone, is not of much quantitative value unless for comparisons under identical conditions, but is often useful as a qualitative method for distinguishing between dextrose (or other monosaccharide) and those disaccharides which also reduce Fehling's solution.

*Reagent.*—Dissolve 45 grams of neutral crystallized cupric acetate in 900 c.c. of water, filter if necessary; add 1.2 c.c. of 50 per cent. acetic acid and dilute to a liter. A portion of this reagent heated in a water-bath must show no change.

*Test.*—To 5 c.c. of this reagent in a test-tube add 5 c.c. of the solution to be tested and place in a boiling water-bath for 3½ minutes; examine for cuprous oxide, viewing the tube against a black background in a good light. If no evidence of reduction is found,

* Barfoed: *Ztschr. anal. Chem.*, 1873, **12**, 27. Müller and Hagen: *Arch. ges. Physiol.* (*Pflüger*), **22**, 325. Lieben: *Ztschr. Vereins Rübenzucker-Industrie*, 1884, **34**, 857; Wiley, Agricultural Analysis, Vol. III., p. 291.

allow the tube to stand at room temperature for 10 minutes and examine again.

*Notes.* — Under the conditions given maltose and lactose do not reduce the reagent. Longer heating or the use of a more acid solution causes a reduction due to the hydrolysis of the disaccharide. In order to prevent this, the solution may be heated to 40° only. At this temperature, however, dextrose reduces very slowly so that the heating must be continued for several hours.

Recent experiments have shown that the test as described is capable of distinguishing between dextrose and maltose in aqueous solutions containing less than 0.02 per cent. of the former and at least 0.2 per cent. of the latter, provided the results are controlled by check experiments made under identical conditions with solutions containing known amounts of dextrose and maltose. Dextrin does not interfere. (Unpublished results by L. J. Cohen.)

If the solution tested contains proteids these are precipitated by the copper, rendering the test slightly less delicate.

In applying the reaction to an unknown solution, comparative tests should be made as directed in the case of the phenylhydrazine test.

### REACTIONS WITH ACIDS.

All carbohydrates on treatment with moderately strong hydrochloric or sulphuric acid yield furfurol, which is probably the cause of the color reaction with α-naphthol described below. Hexa-carbohydrates form only small amounts of furfurol and relatively large amounts of levulinic acid. Penta-carbohydrates yield large amounts of furfurol and no levulinic acid. In all cases, however, there is more or less formation of "humus" and other by-products.

### MOLISCH'S α-NAPHTHOL REACTION.*

Mix 2 to 3 c.c. of the very dilute solution to be tested with 2 or 3 drops of a 15 per cent. solution of α-naphthal in alcohol or chloroform, incline the tube and pour in carefully 2 to 3 c.c. of pure concentrated sulphuric acid. In the presence of carbohydrate, a violet zone appears quickly and spreads by diffusion. If the solution tested contains more than a few milligrams of carbohydrate, it

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* Molisch: *Monatsh. Chem.*, 1886, 7, 198.
Udranszky: *Ztschr. physiol. Chem.*, 1888, 12, 355, 377.
Tollens: Handbuch der Kohlenhydrate, II., 101.
Mulliken: Identification of Pure Organic Compounds, I., 26.

quickly blackens and on dilution with water gives a dull violet precipitate.

A similar test with thymol gives a crimson or carmine-red solution which soon becomes turbid.

FURFUROL TEST FOR PENTOSES AND PENTOSANS.

Place the substance to be tested in an Erlenmeyer flask, add hydrochloric acid (1.06 sp. gr.) and boil. Lay over the mouth of the flask a small filter paper moistened with a solution of anilin acetate.* If the vapor escaping from the flask contains more than traces of furfurol a bright red coloration appears. A test with sucrose or pure starch will show the amount of color to be expected from hexa-carbohydrates.

If the reaction obtained from the unknown substance is much stronger than from sucrose or starch, the presence of pentoses or pentosans is indicated. A few other substances such as glycuronic acid and oxycellulose have been found to yield considerable amounts of furfurol, but these rarely occur in sufficient quantities to require consideration.

DETERMINATION OF PENTOSES AND PENTOSANS.

Under carefully regulated conditions the yield of furfurol from either xylose or arabinose, or from the corresponding anhydride, is nearly constant. If the furfurol is distilled and collected, its amount can be estimated by adding anilin acetate and comparing the red color with that shown by furfurol solutions of known strength.† This method is delicate, but gives only approximate results. Unless the quantity is very small, it should be determined gravimetrically.

Furfurol forms sparingly soluble condensation products with a number of bases and phenols. Phenylhydrazine and phloroglucin have been principally used as precipitants.‡ The Association of Official Agricultural Chemists has adopted provisionally the phloroglucin method in the following form :§

Three grams of the material are placed in a flask, together with

* Prepared by mixing equal volumes of anilin and 50 per cent. acetic acid.

† This colorimetric method is also used for the estimation of furfurol in distilled liquors.

‡ Barbituric acid has recently been recommended as preferable to phenylhydrazine and phloroglucin as a precipitant for furfurol. Unger: Dissertation, Munich, 1904. Jäger and Unger: *Ber. deut. chem. Ges.*, 1902, 35, 4440.

§ Bull. 46, Bur. Chem., U. S. Dept. Agriculture. For a review and discussion of the methods of determining the pentosans and the practical applications of the results see Tollens, Kröber and Rimbach: *Ztschr. angew. Chem.*, 1902, 15, 477, 508. Also

100 c.c. of 12 per cent. hydrochloric acid (sp. gr. 1.06) and several pieces of recently heated pumice stone. The flask, placed upon wire gauze, is connected with a condenser and heat applied, rather gently at first, using a gauze top to distribute the flame, which is so regulated as to distill 30 c.c. in about ten minutes. The 30 c.c. driven over are replaced by a like quantity of the dilute acid, and the process continued so long as the distillate gives a pronounced reaction with anilin acetate on filter paper. To the completed distillate is gradually added a quantity of phloroglucin (free from diresorcin) dissolved in 12 per cent. hydrochloric acid, and the resulting mixture thoroughly stirred. The amount of phloroglucin used should be about double that of the furfurol expected. The solution turns first yellow, then green and very soon an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. The solution is made up to 500 c.c. with 12 per cent. hydrochloric acid, and allowed to stand over night.

The amorphous black precipitate is filtered into a tared gooch through an asbestos felt, washed with 100 c.c. of water, dried to constant weight by heating from three to four hours at 100°, cooled and weighed, the increase in weight being reckoned as phloroglucid. To calculate the furfurol from the phloroglucid, the following formulæ are used:

Phloroglucid (less than 0.2 gram) $\div$ 1.82 = furfurol.
Phloroglucid (from 0.2 to 0.3 gram) $\div$ 1.895 = furfurol.
Phloroglucid (from 0.3 to 0.4 gram) $\div$ 1.92 = furfurol.
Phloroglucid (above 0.4 gram) $\div$ 1.93 = furfurol.

To the weight of furfural thus found, 0.0104 gram is added as a correction for solubility and the corrected weight is multiplied by 1.91 for xylose, 2.35 for arabinose or 2.13 for "pentose."

*Qualitative test of the purity of the phloroglucin.*— Dissolve a small quantity of the phloroglucin in a few drops of acetic anhydrid, heat almost to boiling and add a few drops of concentrated sulphuric acid. A violet color indicates the presence of diresorcin. A phloroglucin which gives more than a faint coloration must be rejected.

### Levulinic Acid Reaction of Hexa-carbohydrates.

According to Wehmer and Tollens,* all hexa-carbohydrates yield levulinic acid in sufficient quantity for identification when treated as follows:

Heat 5 to 20 grams of the substance with 100 c.c. of hydro-chloric acid (1.10 sp. gr.) for 18 hours on a boiling water-bath; ilter the solution, shake with ether to extract the levulinic acid, ınd convert the latter into the zinc or silver salt for identification. As this method is purely qualitative and is not often used in ordi-ıary analytical work, reference must be made to the original paper or details of manipulation.

OXIDATION BY NITRIC ACID.

By heating with moderately strong nitric acid, xylose and ara-›inose are oxidized, each yielding a trioxyglutaric acid, COOH-CHOH)$_3$COOH, while aldoses of the hexose group yield the corresponding acids, COOH(CHOH)$_4$COOH, saccharic, man-ıosaccharic and mucic acids being obtained respectively from lextrose, mannose and galactose.

All of these oxidation products are freely soluble except mucic ıcid, which is practically insoluble in water or dilute nitric acid. Since mucic acid is produced in fairly constant proportion, its in-solubility affords a means for the approximate determination of galactose or any substance which yields galactose on hydrolysis.

*Mucic Acid Method for Galactose, Lactose, Raffinose and Galactans.**

Weigh 1 to 3 grams of substance according to the amount of nucic acid expected; remove fat if necessary by washing with ether; transfer to a beaker about 5.5 cm. in diameter and 7 cm. leep; add 60 c.c. of nitric acid of 1.15 sp. gr. and evaporate the solution to exactly one third its volume on a water-bath at a tem-›erature of 94° to 96°. After standing 24 hours, add 10 c.c. of vater to the precipitate and allow it to stand another 24 hours. The mucic acid has now crystallized, and, unless contaminated vith insoluble residue from the sample, it can be transferred to a veighed filter, washed with 30 c.c. of water, then with alcohol and ether, dried at 100° and weighed.

In case other insoluble substances are present, return the filter nd mucic acid, after washing with water, to the beaker; warm 15 ninutes with a mixture of 1 part strong ammonia, 1 part ammo-ıium carbonate and 19 parts of water; filter and wash; evaporate iltrate to dryness over a water-bath; add 5 c.c. nitric acid of 1.15 sp.

* Tollens and Rischbiet: *Ber. deut. chem. Ges.*, 1885, 18, 2616. Creydt: *Ibid.*, 886, 19, 3115. Bull. 46, Bur. Chem., U. S. Dept. Agriculture.

gr.; stir thoroughly and allow to stand for 30 minutes. Collect the mucic acid on a weighed filter, wash with 10 to 15 c.c. of water, then with 60 c.c. of alcohol and a number of times with ether; dry at 100° and weigh.

When these directions are strictly followed galactose yields about three fourths its weight of mucic acid. The weight of mucic acid obtained from lactose, raffinose or galactan is calculated as three fourths the weight of galactose which could be obtained by hydrolysis. The yield of mucic acid is, however, considerably influenced by the details of manipulation. In carrying out the method a comparative determination with pure milk sugar should always be made, as substances may be present which prevent the crystallization of the mucic acid.*

## Hydrolysis by Dilute Acids.

Monosaccharides, as the name implies, cannot be hydrolyzed to simpler sugars. As a rule, they are unaffected by dilute acids except on prolonged heating, when they are gradually attacked, yielding in part decomposition products like those produced by stronger acids, and undergoing a partial "reversion" with the formation of di- or polysaccharides. Dextrose, for example, when heated too long with dilute hydrochloric or sulphuric acid, is changed partially to "isomaltose" and to dextrin-like anhydrides, such as "gallisin," the unfermentable constituent of crude commercial glucose. Levulose decomposes much more readily than dextrose on heating with dilute acids.

Disaccharides differ considerably in the readiness with which they are hydrolyzed by acids. Sucrose is very easily hydrolyzed, a 20 per cent. solution being completely changed to invert-sugar by mixing with one-tenth its volume of concentrated hydrochloric acid (making about 3 per cent. of actual acid in the mixture) and warming to 68° at such a rate as to require 15 minutes heating.†

Maltose is less easily hydrolized than sucrose, a 2 per cent. solution in 2 to 3 per cent. hydrochloric acid requiring 30 to 40 minutes' boiling, or 2 to 3 hours' heating on a water bath, for com-

* Herzfeld: *Ztschr. Vereins Rübenzucker Ind.*, 1890, 40, 265; Abs. *Chem. Ztg.*, 1890, 14 *Rep.*, 108. Stone and Baird: *Jour. Amer. Chem. Soc.*, 1897, 19, 119.

† Clerget's method, described by Wiley: Agricultural Analysis, Vol. III., pp. 105–107. According to Borntrâger and to Samelson (*Ztschr. angew. Chem.*, 1892, 334; 1893, 690; 1894, 267, 351), the sucrose in such a mixture is completely hydrolyzed by standing over night at room temperature.

plete hydrolysis to dextose.* Lactose is also less readily hydrolyzed than sucrose, the difference being especially marked in the case of weak acids. By boiling with citric acid, sucrose can be completely converted to invert-sugar, while lactose remains unchanged. (See methods for sucrose and lactose given later.)

Raffinose is hydrolyzed by dilute mineral acids, slowly in the cold, much more rapidly on boiling, requiring in either case more vigorous treatment than does sucrose.

Dextrin, glycogen and starch are hydrolyzed to dextrose under the same conditions as maltose, but require somewhat longer heating. Pentosans and galactans (at least the more common forms) are hydrolyzed by acids almost as readily as starch. Normal cellulose is not hydrolyzed by boiling dilute acids. Hemicellulose is a term commonly applied to the carbohydrate matter in the cell walls of plants, more resistant to enzymes than starch, but dissolved and hydrolyzed on boiling with dilute mineral acids. The hemicellulose of the common food plants consists largely of pentosans.

## ROTATION OF POLARIZED LIGHT.

Solutions of the natural carbohydrates have the property of rotating the plane of polarized light. This rotating power is also shown by most other natural substances containing one or more asymmetric carbon atoms in the molecule.

### Measure of Rotating Power — Specific Rotation.

The first organic substances to be studied by means of polarized light were liquids, or solutions of known density. In order to reduce the observations to a common basis for comparison Biot introduced the following formula :

$$[a] = \frac{a}{l \times d}$$

in which $[a]$ = the "specific rotating power"

$a$ = the observed angular degrees of rotation

$l$ = the length in decimeters of the column of liquid traversed by the polarized light

$d$ = the density of the liquid.

* It is sometimes stated that maltose cannot be made to yield more than about 98 per cent. of the theoretical amount of dextrose, the acid always beginning to attack the latter before the hydrolysis of the former is quite complete. See notes on the determination of starch.

On extending the calculation to solids in solution the above formula becomes

$$[\alpha] = \frac{100\ a}{l \times d \times p}$$

in which $p =$ the percentage of solid in the solution.

But since $d \times p$ equals the number of grams of solid in 100 c.c. of solution it is simpler to write the formula

$$[\alpha] = \frac{100\ a}{l \times c}$$

in which $c =$ concentration, *i. e.*, the number of grams of dissolved solid in 100 c.c. of the solution.

The value of the specific rotating power $[\alpha]$ is, therefore, the number of angular degrees through which a ray of polarized light would be rotated in traversing one decimeter of a solution of which each cubic centimeter contained one gram of the active substance.

The angular rotation is always directly proportional to the length of the column of liquid through which the light passes. It always depends upon the wave length of the light ray employed. By measuring the rotation for different rays the rotation-dispersion of the substance is determined. Sodium light corresponding to the Fraunhofer line *D* is commonly used in determining the rotating power. In many cases the rotating power is appreciably influenced by temperature. The latter should therefore be stated in giving the value of $[\alpha]$. Thus $[\alpha]_D^{20}$ indicates the rotating power as measured with *D* light at 20°. The concentration of the solution has usually an appreciable influence upon the value of $[\alpha]$ as is shown by the following statements of rotating power of the more important carbohydrates:

Dextrose (Tollens)*

$$[\alpha]_D^{17} =† 52.50 + 0.018796p + 0.0005168p^2.$$

Levulose (Jungfleish and Grimbert)‡

$$[\alpha]_D^t = -[101.38 - 0.56t + 0.108(c - 10)].$$

Galactose (Meissl)§

$$[\alpha]_D^t = 83.883 + 0.0785p - 0.209t.$$

* *Ber. deut. chem. Ges.*, 1876, 9, 487, 1531; 1884, 17, 2234.

† Unless otherwise stated the rotation is to the right (+); lævorotation is indciated by (—).

‡ *Compt. rend.*, 1888, 107, 390.

§ *Journ. prakt. Chem.*, 1880, [2], 22, 97.

Sucrose (Tollens) *

$$[\alpha]_D^{20} = 66.386 + 0.015035p - 0.0003986p^2.$$

Lactose (Schmöger)†

$$[\alpha]_D^{20} = 52.53 \text{ (constant for } c = 2.4 \text{ to } 40).$$

Maltose (Meissl)‡

$$[\alpha]_D^t = 140.375 - 0.01837p - 0.095t.$$

At ordinary concentrations and laboratory temperatures, the values for $[\alpha]_D$ are approximately:

| | | | |
|---|---|---|---|
| Dextrose | 53 | Sucrose | 66.5 |
| Levulose | $-[102 - .56t]$ | Lactose | 52.5 |
| Invert-sugar | $-[27.9 - .32t]$ | Maltose | 138. |
| Galactose | 80.5 | Raffinose | 104.5 |
| Mannose | 13 | Starch } Glycogen } | 190 to 210 |
| Arabinose | 104 | | |
| Xylose | 19 | Dextrins | 160 to 210 |

PREPARATION OF SOLUTIONS FOR POLARIZATION.

Reducing sugars when first dissolved often show rotating powers varying greatly from those given. This property, known as multirotation, or birotation, occasionally causes errors in analytical work. The normal rotation is established by allowing the solution to stand for some hours, by boiling, or by the addition of about 0.1 per cent. of ammonia. Solutions containing ammonia darken on standing and should therefore be polarized as soon as prepared.

Sucrose does not show multirotation, and is not appreciably affected by ordinary changes of temperature, while the changes of rotating power due to variations in concentration are small and have been accurately determined.

Solutions for polarization must be clear and free from all impurities having an influence upon polarized light. Among the optically active substances most likely to be met in natural products are organic acids (especially tartaric), pectin bodies, gums, resins, coloring matters, glucosides, alkaloids and proteids. In most cases the interfering substances can be precipitated and the solution clarified by adding, first, basic lead acetate, and then a cream of aluminium hydroxide. It is sometimes advantageous to add a small amount of tannin before clarifying. Tannin combines with many of the substances mentioned, either precipitating them or forming

* *Ber. deut. Chem Geo.*, 1877, 10, 1403.

† *Ibid.*, 1880, 13, 1922.

‡ *Journ. prakt. Chem.*, 1882, [2], 25, 114.

compounds more easily precipitated by the basic acetate. In case the solution, after being treated with clarifying agents, made up to definite volume and filtered, is too highly colored for examination in the polariscope, it may be poured slowly through a small amount of pure dry animal charcoal on a paper filter. Since the charcoal absorbs a small amount of the sugar, the first portions of the filtrate must be rejected.

### Determination of Angular Rotation.

For the direct determination of $[\alpha]_D$, sodium light must be used. The instrument most commonly employed is the Laurent polariscope. This has a circular scale graduated in angular degrees. The scale is divided into half-degrees and the vernier is graduated to read minutes of angular rotation. With this polariscope the rotation produced by the active substance is found by turning the analyzing prism, which is fixed in position with regard to the scale but rotates on its axis. The zero point is found with the polariscope empty and is the point at which the crystal axis of the analyzing prism is at right angles to the plane of polarization. If now an active substance is placed between the polarizing and analyzing prisms, the latter must be rotated until it stands at right angles to the new plane, when the appearance of the field will be the same as that previously observed at the zero point. The position of the scale now shows directly the number of angular degrees through which the plane of polarization has been rotated by the active substance.

For the examination of sugar solutions, especially in technical analysis, the Schmidt and Haensch saccharimeter with Ventzke scale is commonly used. This instrument, which is made in several forms, is different in principle from the Laurent polariscope. White light is used and the rotation, instead of being measured directly, is "compensated" by means of quartz wedges, the thickness of quartz required to neutralize the effect of the active substance giving a measure of the rotation produced by the latter. With the trough of the saccharimeter empty, the scale should read zero when the quartz wedges are so placed that the light passes through equal thicknesses of positive (dextrorotatory) and negative (lævorotatory) quartz. If now a tube containing a solution of sucrose is introduced, the thickness of negative quartz is increased, or that of positive quartz diminished, until the rotation produced

by the sugar solution is compensated by the opposite rotation of the quartz. This method of measuring the rotation by means of quartz with white light illumination is applicable only to such active bodies as have practically the same rotation dispersion as quartz. This is true of aqueous sugar solutions, which can therefore be examined by either of the instruments mentioned. The readings are compared by assuming that each degree of the Ventzke sugar scale is equivalent to 0.3468 degrees of angular rotation.

For descriptions of apparatus and details of testing and manipulation, the standard works on sugar analysis should be consulted. A number of the more important of these are among the reference books listed below, but attention may be especially directed to the work of Landolt as containing the most exhaustive and authoritative discussion of polarized light and its analytical applications.

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## CHAPTER VI.

### Carbohydrates—Special Methods.

#### ANALYSIS OF RAW SUGAR.

#### POLARISCOPIC EXAMINATION.

A special room of even temperature which can be darkened when desired should be provided for the polariscopic examination. For illumination of the white-light saccharimeter, a triple flame or an Argand burner with incandescent mantle is used. In *taking readings the polariscope must be brought only near enough* to the burner to secure a good illumination of the field, never within less than eight inches. As soon as the reading is taken the polariscope should be turned away or the flame lowered, in order to avoid any possible warming of the instrument.

*Determination of the Zero Point.*—The trough of the saccharimeter being empty, set the scale within a few degrees of zero and focus the eyepiece so that the field of vision is clear and the perpendicular line or band dividing it is perfectly distinct. Now rotate the milled head so as to move the zero point of the scale toward that of the vernier. When the neutral point (the true zero point for the instrument as it stands) is reached the appearance of the entire field is uniform. Approach the zero point first from one side then from the other, taking the reading each time as soon as the field appears uniform, until successive readings do not differ by more than 0.2° on the Ventzke sugar scale. The average of ten such readings is taken as the zero point in all subsequent work with the instrument unless it should be jarred or moved, in which case the zero point should be redetermined.

*Test with Pure Sugar.*—Weigh 26* grams of pure sucrose, dis-

* The value of the Ventzke scale was originally fixed by means of pure sucrose solutions of 1.100 sp. gr. at 17.5° and it was found that 100 c.c. of such a solution contain 26.048 grams of sucrose (weighed in air with brass weights). For many years, however, it has been the custom of instrument makers to calibrate the Ventzke scale by means of solutions containing 26.048 grams of sucrose in 100 Mohr cubic centimeters. A solution of very nearly the same strength is obtained by dissolving 26 grams and completing the volume at 20° to 100 metric cubic centimeters (the volume occupied by 100 grams of water at 4° weighed in vacuo). The latter proportions have recently been adopted by the International Commission for Unifying Methods of Sugar Analysis.

solve in water in an accurately calibrated 100 c.c. flask, fill to the mark at 20° to 22° and mix throughly by shaking, holding the flask in such a way as not to warm the solution. The latter should have a density very nearly 1.10 and should rotate the plane of polarized light 34.68° to the right, giving a reading of 100.0° on the Ventzke scale.

Rinse the 200 mm. tube several times with the solution, then fill until the curved surface of the liquid projects above the open end of the tube; see that all air-bubbles have risen to the surface and then slide on the cover-glass horizontally in such a manner that the excess of liquid is carried over the side, leaving the cover-glass exactly closing the tube, with no air-bubbles beneath it and none of the liquid upon its upper surface. The cover-glass being in position, the tube is closed by screwing on the cap. The latter should be only tight enough to prevent leakage, as any considerable pressure on the glass plate may cause it to become optically active.

Place the tube in the trough of the saccharimeter or polariscope and take ten readings as in setting the zero point. The average reading (corrected for zero point) should not differ from 100.0° by more than 0.2° on the Ventzke scale, nor from 34°41′ on the Laurent polariscope by more than 5′.

*Polarization of the Raw Sugar.*—Mix the sample, weigh out 26 grams and dissolve in 60 to 80 c.c. of water in a 100 c.c. flask. When the sugar is entirely dissolved, add from 1 to 5 c.c. (according to the nature of the sample; only very dark sugars should require more than 2 c.c.) of a solution of basic lead acetate of about 1.25 sp. gr.* A decided excess of basic acetate should never be used. After the lead acetate has been added and mixed, add twice its volume of "alumina cream." † This assists in the clarification, precipitates the excess of lead, and facilitates filtration. A moderate excess of alumina cream does no harm. With high grade

* This may be prepared by dissolving the solid basic salt or by boiling an excess of litharge with a strong solution of neutral lead acetate.

† Prepared as follows: Shake powdered commercial alum with water at ordinary temperature until a saturated solution is obtained. Set aside a little of the solution, and to the residue add ammonia, little by little, stirring between additions, until the mixture is alkaline to litmus paper. Then drop in additions of the portion left aside, until the mixture is just acid to litmus paper. By this procedure a cream of aluminium hydroxide is obtained suspended in a solution of ammonium sulphate. This sulphate is advantageous when added after the basic acetate since it precipitates whatever excess of lead may be present.

sugars the use of alumina cream alone may be sufficient for clarification. Make up to volume with distilled water,* shake well and then pour the whole solution, or as much as practicable, on a dry filter. Reject the first 20 or 30 c.c. of filtrate and then polarize the remainder as already described for the pure sugar solution The average reading of the Ventzke scale, corrected for the deviation of the zero-point, is reported as "polarization."

*Notes and Precautions.* — Care must be taken to avoid the following errors due to manipulation; (1) change in moisture content of sample during weighing, (2) change in volume of solution due to fluctuations of temperature, (3) imperfect mixing of solution after diluting to volume in the graduated flask, (4) evaporation during filtration, (5) too great compression of cover glasses in closing the polariscope tube.

Clarification is very important. All proteids are lævoratory and must be entirely removed. The solution must be free from turbidity, but not necessarily free from color. Only so much of the clarifying agents should be used as is necessary to free the solution from optically active impurities and from turbidity. Any excess increases the error due to the presence of precipitate when the solution is diluted to volume. The volume occupied by the precipitate varies between 0.05 and 1.0 c.c. for ordinary raw sugars. It can be determined by Scheiber's method of double dilution † in which a duplicate determination is made in a flask of twice the volume, or by determining the weight and density of the precipitate as recommended by Sachs. ‡ The latter method is preferred by Wiechmann § and Horne ‖ each of whom determined the volume of the precipitate for a number of raw sugars from different localities. Horne has found (*loc. cit.*) that the error can be almost entirely avoided by clarifying with anhydrous basic acetate after the sugar solution has been diluted to volume.

### *Regulations of the International Commission.*

The following resolutions ¶ adopted by the International Commission for the Unification of Sugar Analysis in 1900, have since been generally accepted.

* If frothing interferes at this point, add two or three drops of ether.

† *Ztschr. Vereins. Rübenzucker-Industrie*, 1875, 25, 1054.

‡ *Ibid.*, 1880, 30, 229. See also the paper by Horne.

§ *Ibid.*, 1903, [n. f.]40, 498; Abs. *Journ. Chem. Soc.*, 1903, 84, ii, 699.

‖ *Journ. Amer. Chem. Soc.*, 1904, 26, 186.

¶ U. S. Dept. Agriculture, Bur. Chem., Bull. 73, p. 58. *Journ. Amer. Chem. Soc.*, 1901, 23, 59.

1. In general, all sugar tests shall be made at 20° C.

2. The graduation of the saccharimeter shall be made at 20° C. Twenty-six grams of pure sugar, dissolved in water, and the volume made up to 100 metric cubic centimeters, or during the period of transition 26.048 grams of pure sugar in 100 Mohr cubic centimeters, all weighings to be made in air with brass weights, the completion of the volume and the polarizations to be made at 20° on an instrument graduated at 20°, should give an indication of 100 on the scale of the saccharimeter. For countries where temperatures are usually higher than 20°, it is permissible that saccharimeters be graduated at 30°, or any other suitable temperature, under the conditions specified above, providing that the analysis of the sugar be made at the same temperature — that is, that the volume be completed and the polarization made at the temperature specified.

3. Preparation of pure sugar: Purest commercial sugar is to be further purified in the following manner: A hot saturated aqueous solution is prepared and the sugar precipitated with absolute ethyl alcohol; the sugar is carefully spun in a small centrifugal machine and washed in the latter with absolute alcohol. The sugar thus obtained is re-dissolved in water, the saturated solution again precipitated with alcohol and washed as above. The product of the second crop of crystals is dried between blotting paper and preserved in glass vessels for use. The moisture still contained in the sugar is determined and taken into account when weighing the sugar which is to be used.

The committee further decided that central stations shall be designated in each country which are to be charged with the preparation and distribution of chemically pure sugar. Wherever this arrangement is not feasible, quartz plates, the values of which have been determined by means of chemically pure sugar, shall serve for the control of the saccharimeters.

The committee further decided that the above control of quartz plates by means of chemically pure sugar should, as a rule, apply only to the central stations which are to test the correctness of saccharimeters; for those who execute commercial analyses, the repeated control of the instruments is to be accomplished, now as before, by quartz plates.

4. In effecting the polarization of substances containing sugar, half-shade instruments, or triple field, only are to be employed.

5. During the observation the apparatus must be in a fixed position and so far removed from the source of light that the polarizing nicol is not warmed.

6. Sources of light may be gas, triple burner with metallic cylinder, lens and reflector; gas lamp with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; sodium light. Several readings are to be made and the mean thereof taken, but any one reading must not be neglected.

7. In making a polarization the whole normal weight for 100 cubic centimeters is to be used, or a multiple thereof for any corresponding volume.

8. As clarifying and decolorizing reagents there may be used: (*a*) subacetate of lead, (3 parts by weight of acetate of lead, one part by weight of oxide of lead, 10 parts by weight of water); (*b*) alumina cream; (*c*) concentrated solution of alum. Boneblack and decolorizing agents are to be excluded.

9. After bringing the solution exactly to the mark, at the proper temperature, and after wiping out the neck of the flask with filter paper, all of the well-shaken clarified sugar solution is poured upon a dry rapidly-acting filter. The first portions of the filtrate are to be rejected and the rest, which must be perfectly clear, used for polarization.

*Relation of Polarization to Percentage of Sucrose.*

In the absence of other active substances, the reading of the Ventzke scale as obtained above shows the percentage of sucrose in the sample. In commercial raw sugar the only other sugars likely to be present are dextrose and levulose. If the total amount of these reducing sugars, as shown by Fehling's solution, is small, the value of the sample is sufficiently indicated by the polarization, the invert-sugar, and the amounts of moisture and ash.

Since, however, the "reducing sugar" contains dextrose and levulose in unknown proportions, the determination of "reducing power" does not give the necessary data for estimating the percentage of sucrose in the sample. The percentage of sucrose can, however, be determined in the presence of a known mixture of dextrose and levulose by observing the change in rotation produced by hydrolysis. This is unaffected by the presence of monosaccharides since the latter cannot be hydrolyzed.

The following method is based on this principle:

## Clerget's Method for Sucrose.

A pure sucrose solution polarizing 100° on the Ventzke scale at a temperature of 20°, will, after hydrolysis, polarize — 33.6° on the same scale at the same temperature. It is this change of rotation from right to left which gives rise to the terms "inversion" for hydrolysis, and "invert-sugar" for the mixture of equal parts of dextrose and levulose produced. Since the lævorotatory power of levulose, and hence of invert-sugar, decreases rapidly with increase of temperature the reading 33.6° would be found only at 20°. An increase of 1° in temperature causes a decrease of 0.5 degree Ventzke in the lævorotation of the invert-sugar, and at about 87.2° the reading becomes zero. The change in rotation produced by hydrolysis in pure sucrose solution is, therefore, $143.6 - 0.5t$, in which $t$ is the temperature at which the readings are taken.

To estimate the sucrose in an unknown sample, dissolve 26 grams, clarify, dilute to 100 c.c., filter and polarize as usual. Place 50 c.c. of the filtrate in a flask marked at 50 and 55 c.c., fill to the 55 c.c. mark with pure concentrated hydrochloric acid and mix well. Set the flask in water and heat until a thermometer suspended with the bulb at the center of the solution reads 68°, regulating the heat so that about 15 minutes are consumed in reaching the required temperature. Remove the flask, cool quickly, and polarize in a 220 mm. tube at exactly the temperature at which the first reading was taken. The change of rotation divided by that which pure sucrose would show under the same conditions, gives the percentage of sucrose in the sample. Or stating this in the form of an equation

$$\text{Sucrose} = \frac{a - b}{143.6 - 0.5t}$$

in which $a$ is the first reading, $b$ the second reading and $t$ the temperature.

*Notes.* — In the above description the manipulation of Clerget and the figures found by Borntrager * and adopted by Wiley † have been followed. Landolt adopts other conditions requiring a different factor, 142.4 instead of 143.6. The temperature of each polarization must be carefully noted. Since the solution is increased one-tenth in volume by the addition of hydrochloric acid, the sec-

* *Ztschr. Vereins Rubenzucker Ind.*, 1890, 876.

† Agricultural Analysis, Volume III., 108.

ond reading must be increased one tenth or made in a tube one tenth longer than that first used.

The Clerget principle can be applied to the determination of sucrose in the presence of any monosaccharide or mixture of monosaccharides. By the use of citric, in place of hydrochloric, acid it can also be used for mixtures containing lactose.

### Determination of Reducing Sugar.

Usually the reducing power of the raw sugar is determined volumetrically, as described in the preceding chapter, the results being calculated as percentage of invert-sugar. For the determination dissolve 5 grams of the sample if dark, or 10 grams if light, dilute to 100 c.c. and filter through dry paper. If the preliminary test shows the solution to be too strong or too weak, prepare a fresh solution for the final determination. When the volumetric method is carefully carried out, the results are sufficiently exact for all ordinary work.* For the determination of very small amounts of invert-sugar the method introduced by Herzfeld,† and recommended by the International Commission,‡ is probably the most accurate.

#### *Herzfeld's Gravimetric Method.*

Dissolve 20 grams of sample in 80 c.c. of water, clarify with basic lead acetate and precipitate the excess of lead by sodium carbonate § (avoiding more than a slight excess) or by neutral potassium oxalate;‖ dilute to 100 c.c., mix thoroughly and filter through dry paper. The filtrate must be perfectly clear. In a beaker of 250 c.c. capacity, place 50 c.c. of the undiluted "mixed Fehling reagent" (see Fehling's volumetric method) and 50 c.c. of the clarified sugar solution; heat at such a rate that the mixture boils in about four minutes, and boil for exactly two minutes. Add 100 c.c. of cold, recently boiled, distilled water, filter immediately, and find the amount of copper reduced as described under Defren's method in the preceding chapter. The following table shows the corresponding percentage of invert-sugar.

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* If a gravimetric method is preferred, use that of Meissl and Hiller, as adopted by the Official Agricultural Chemists: Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

† *Ztschr. Vereins Rubenzucker Industrie*, 1886, 6.

‡ *Journ. Amer. Chem. Soc.*, 1901, 23, 64.

§ Bul. 46, *loc. cit.*, p. 33.

‖ Sawyer: *Journ. Amer. Chem. Soc.*, 1904, 26, 1631.

HERZFELD'S TABLE FOR THE DETERMINATION OF INVERT SUGAR IN MATERIALS CONTAINING 1 PER CENT. OR LESS OF INVERT SUGAR AND A HIGH PERCENTAGE OF SUCROSE.

| Copper reduced by 10 grams of material. Milligrams | Invert sugar. Per cent. | Copper reduced by 10 grams of material. Milligrams. | Invert sugar. Per cent. | Copper reduced by 10 grams of material. Milligrams. | Invert sugar. Per cent. |
|---|---|---|---|---|---|
| 50 | 0.05 | 120 | 0.40 | 190 | 0.79 |
| 55 | 0.07 | 125 | 0.43 | 195 | 0.82 |
| 60 | 0.09 | 130 | 0.45 | 200 | 0.85 |
| 65 | 0.11 | 135 | 0.48 | 205 | 0.88 |
| 70 | 0.14 | 140 | 0.51 | 210 | 0.90 |
| 75 | 0.16 | 145 | 0.53 | 215 | 0.93 |
| 80 | 0.19 | 150 | 0.56 | 220 | 0.96 |
| 85 | 0.21 | 155 | 0 59 | 225 | 0.99 |
| 90 | 0.24 | 160 | 0.62 | 230 | 1.02 |
| 95 | 0.27 | 165 | 0.65 | 235 | 1.05 |
| 100 | 0.30 | 170 | 0.68 | 240 | 1.07 |
| 105 | 0.32 | 175 | 0.71 | 245 | 1.10 |
| 110 | 0.35 | 180 | 0.74 | | |
| 115 | 0.38 | 185 | 0.76 | | |

## DETERMINATION OF MOISTURE AND ASH.

Dry about 2 grams of sample in a flat-bottomed platinum dish in the boiling water oven until the loss in weight on heating for one hour does not exceed 0.10 per cent. A perfectly constant weight cannot be expected if invert-sugar is present, since levulose is slowly decomposed by heating at 100° in the air. Calculate the loss of weight as moisture.

Moisten the residue with a few drops of concentrated sulphuric acid and burn to whiteness. Weigh; and report the result as "sulphated ash." The true ash is considered by some as nine-tenths and by others as four-fifths of the "sulphated ash."

## OFFICIAL METHODS AND STANDARDS OF PURITY.

Methods of the Association of Official Agricultural Chemists: Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

Regulations governing the sampling and classification of imported sugars and molasses: Treasury Department Document No. 2113.

German official methods: *Ztschr. Unters. Nahrungs- und Genussmittel*, 1903, **6**, 1059; *Ztschr. anal. Chem.*, 1903, **42** (Appendix to Nos. 9 and 10).

An act of Congress approved June 3, 1902, authorized the Secretary of Agriculture to establish standards of purity for food

products. The definitions and standards established for sugar and sugar products are as follows: *

1. *Sugar* is the product chemically known as sucrose (saccharose) chiefly obtained from sugar cane, sugar beets, sorghum, maple, or palm.

*Standard sugar* is white sugar containing at least ninety-nine and five-tenths (99.5) per cent. of sucrose.

2. *Granulated, loaf, cut, milled,* and *powdered sugars* are different forms of standard sugars.

3. *Maple sugar* is the solid product resulting from the evaporation of maple sap.

4. *Massecuite, melada, mush sugar,* and *concrete* are products obtained by evaporating the purified juice of a sugar-producing plant, or a solution of sugar, to a solid or semi-solid consistence in which the sugar chiefly exists in a crystalline state.

5. *Molasses* is the product left after separating the sugar from massecuite, melada, mush sugar, or concrete.

*Standard molasses* is molasses containing not more than twenty-five (25) per cent. of water nor more than five (5) per cent. of ash.

6. *Sirup* is the product obtained by purifying and evaporating the juice of a sugar-producing plant without removing any of the sugar.

7. *Sugar-cane sirup* is a sirup obtained by the evaporation of the juice of the sugar cane or by the solution of sugar-cane concrete.

8. *Sorghum sirup* is a sirup obtained by the evaporation of sorghum juice or by the solution of sorghum concrete.

9. *Maple sirup* is a sirup obtained by the evaporation of maple sap or by the solution of maple concrete.

10. *Sugar sirup* is a product obtained by dissolving sugar to the consistence of a sirup.

*Standard sirup* is a sirup containing not more than thirty (30) per cent. of water nor more than two and five-tenths (2.5) per cent. of ash.

DETERMINATION OF SUCROSE IN BEETS AND CANE.

Sucrose is the only sugar found in appreciable quantity in the fresh juice of healthy sugar beets. Sugar cane juice contains a small amount of reducing sugar which is usually considered to be invert-sugar, but which according to Wiley † is without influence upon polarized light. In either case therefore the polarization of

* U. S. Dept. of Agriculture, Office of the Secretary, Circular No. 10 (November, 1903). See also Circular No. 13.

† Agricultural Analysis, Volume III., p. 234. The reducing substances of the original cane juice should not be confused with the invert-sugar produced by hydrolysis of sucrose, in the process of sugar manufacture.

a properly clarified sample is considered as showing the amount of sucrose present.

Beets after being thoroughly cleaned (the loss of weight in cleaning being determined and reported as "tare") are reduced to a fine pulp by rasping or grinding. This pulp is then treated in one of three ways (1) separating the insoluble matter by pressure and polarizing the juice, (2) extracting with alcohol, (3) extracting with water. The first method introduces errors from imperfect separation of the juice, the second is longer and more expensive than the third which is now generally used. If, however, the pulp be treated with water and filled to volume in a graduated flask as in the analysis of raw sugar it is very difficult to expel the air bubbles entirely from the mixture of pulp and water. The following method described by Sachs * avoids this inconvenience and has been found satisfactory for rapid work: Weigh 26 grams of the pulp in a beaker and add 177 c.c. of water containing 5 c.c. of the usual solution of basic lead acetate; shake or stir for three minutes; filter and polarize the filtrate. Twice the reading of the Ventzke scale is approximately the percentage of sucrose in the sample. This method assumes that 26 grams of beet pulp contain 23 c.c. of juice, making the total volume of liquid in the mixture 200 c.c. Many German sugar chemists hold that extraction with water gives high results because the beet contains substances other than sucrose which are soluble in water and rotate polarized light to the right. According to Sachs, however, these substances are pectin-like bodies and are completely precipitated by basic lead acetate. Trowbidge † also found that the results obtained by digestion in water were practically identical with those of the alcohol extraction method.

In sampling sugar cane for analysis, the canes are usually cut into thin oblique chips, "cosettes," by means of rotating knives. These cosettes may be further reduced by passing through a sausage cutter or a special shredding machine. It is not feasible, however, to reduce the cane to as fine a pulp as is obtained from sugar beets. For this reason more time and a higher temperature must be used to ensure complete extraction of the sucrose. According to Wiley, ‡ 26 grams of the chips of pulp are weighed into a

* Paper read before the Second International Congress of Applied Chemistry, Paris, 1896; quoted by Hiltner and Thatcher, *Journ. Amer. Chem. Soc.*, 1901, 23, 300.

† *Journ. Amer. Chem. Soc.*, 1901, 23, 222.

‡ Agricultural Analysis, Vol. III., p. 238.

*flask graduated at 102.6 c.c., this graduation being based on the* assumption that the cane contains ten per cent. of insoluble matter of a specific gravity about equal to that of the solution. The flask is nearly filled with water, and warmed on a water-bath for one hour with frequent shaking; then filled a little above the mark and warmed for ten minutes longer with frequent shaking. The flask is now cooled, the volume adjusted if necessary and the liquid filtered through dry paper. Fifty c.c. of filtrate are collected in a 55c.c. flask, clarified with basic acetate, filled to the mark, shaken, filtered, and the filtrate polarized in a 220 mm. tube.

For further information on the analysis of sugar beets and sugar cane, the following books and papers may be consulted:

Wiley: Agricultural Analysis, Vol. III., Part III.

Spencer: Handbook for Beet Sugar Chemists. Handbook for Sugar Manufacturers.

Lippmann and Pulvermacher: Lunge's Chemisch-technische Untersuchungsmethoden.

Frühling and Schultz: Anleitung zur Untersuchung für die Zuckerindustrie.

Trowbridge: Notes on Sugar Beets, *Journ. Amer. Chem. Soc.*, 1901, **23**, 216.

Hiltner and Thatcher: An Improved Method for the Rapid Estimation of Sugar in Beets, *Journ. Amer. Chem. Soc.*, 1901, **23**, 299, 863.

## COMMERCIAL GLUCOSE.

### Official Definitions and Standards of Purity. *

1. *Starch sugar or grape sugar* is the solid product obtained by hydrolyzing starch or a starch-containing substance until the greater part of the starch is converted into dextrose. Starch sugar or grape sugar appears in commerce in two forms, anhydrous and hydrous. In the former the sugar is crystallized without, in the latter with, water of crystallization. The hydrous varieties are commonly known as 70 and 80 sugars; 70 sugar is also known as brewers' sugar, and 80 sugar as climax or acme sugar.

(*a*) *Standard 70 sugar or brewers' sugar* is hydrous starch sugar containing not less than 70 per cent. of dextrose and not more than 0.8 per cent. of ash.

* U. S. Dept. Agriculture, Office of the Secretary, Circulars 10 and 13.

(*b*) *Standard 80 sugar, climax or acme sugar*, is hydrous starch sugar containing not less than 80 per cent. of dextrose and not more than 1.5 per cent. of ash.

(*c*) *Standard anhydrous grape sugar* is anhydrous grape sugar containing not less than 95 per cent. of dextrose without water of crystallization and not more than 0.8 per cent. of ash.

The ash of these standard products consists almost entirely of chlorides and sulphates.

2. *Glucose, mixing glucose, or confectioners' glucose* is a thick sirupy substance obtained by incompletely hydrolyzing starch or a starch-containing substance, decolorizing and evaporating the product.

*Standard glucose, mixing glucose, confectioners' glucose* is colorless glucose, varying in density between 41 and 45 degrees Baumé, at a temperature of 100 degrees F. It conforms in density, within these limits, to the degree Baumé it is claimed to show, and for a density of 41 degrees Baumé contains not more than 21 per cent. of water, and for a density of 45 degrees not more than 14 per cent. It contains on a basis of 41 degrees Baumé not more than 1 per cent. of ash, consisting chiefly of chlorides and sulphates.

3. *Glucose sirup or corn sirup* is glucose unmixed or mixed with sirup, molasses, or refiners sirup [the latter being defined in Circular No. 13 as the residual liquid product ("treacle") obtained in the process of refining raw sugars].

*Standard glucose sirup or corn sirup* is glucose sirup or corn sirup containing not more than 25 per cent. of water nor more than 3 per cent. of ash.

### Analysis by Wiley's Method.*

This method was devised for the analysis of mixtures resulting from incomplete hydrolysis of starch, and is based on the assumption that dextrose and maltose are oxidized to optically inactive products when heated with an alkaline solution of mercuric cyanide and that dextrin is unaffected by the treatment and shows a specific rotation of + 193.

Dissolve 10 grams of sample in 60 to 70 c.c. of water in a 100 c.c. flask; boil for 10 to 15 minutes to secure a normal rotation of the reducing sugars; cool, add some alumina cream, if the solution

* *Chem. News*, 1882, 46, 175; Agricultural Analysis, Vol. III., pp. 286–293.

is not clear, and fill to the mark. After thorough mixing, filter and polarize in a 200 mm. tube. Calculate the result as apparent specific rotation of the original sample.

To 10 c.c. of the same filtrate add 50 c.c. of the cyanide solution* and boil for 3 to 5 minutes. Add hydrochloric acid to faint excess, when the brown color of the solution will disappear; cool, and dilute the solution to 100 c.c. Filter (through animal charcoal if necessary) and polarize in the same instrument as before. This result is calculated to apparent specific rotation as before, basing the calculation on the entire amount of the original substance.

Dilute 10 c.c. of the filtrate first obtained to 100 c.c. and determine the reducing power by Defren's method (preceding chapter), or by Allihn's method, as given under determination of starch. Calculate the reducing power as percentage of dextrose in the original sample.

Let $P$ = the first polarization calculated as apparent specific rotation (due to dextrose, maltose and dextrin).

$P'$ = the second polarization calculated as apparent specific rotation (due to dextrin).

$R$ = the reducing power calculated as dextrose.

$d$ = dextrose.

$m$ = maltose.

$d'$ = dextrin.

Assuming the reducing power of maltose to be 0.61 that o dextrose, and the value $[\alpha]_D$ to be respectively, dextrose, 53 maltose, 138; dextrin, 193:

$$P = 53d + 138m + 193d'.$$
$$P' = 193d'.$$
$$R = d + 0.61m.$$

Solve for $d$, $m$ and $d'$ to obtain the amounts of dextrose maltose and dextrin in the original sample.

### Analysis by Other Methods.

Several other methods for the analysis of commercial glucos have been proposed. Some of these do not provide for the est

* Prepared by dissolving 120 grams of mercuric cyanide in water, adding 120 gran of sodium hydroxide and diluting the solution to 1 liter.

mation of maltose, but attribute the entire reducing power to dextrose. Others, including that of Allen, assume that dextrin, maltose and dextrose are the only organic compounds present. The percentage of solids is usually estimated from the specific gravity, the ash determined and deducted. Assuming that the total percentage of dextrose, maltose and dextrin is now known, the amount of each can be found by one determination of the apparent specific rotation and a determination of the reducing power. For details of the methods consult Allen's Commercial Organic Analysis, Volume I. (third edition), pages 358–378, and the following papers:

Brown, Morris and Millar: Experimental Methods Employed in the Examination of the Products of Starch Hydrolysis, *Journ. Chem. Soc.*, 1897, **71**, 72.

Rolfe and Defren: An Analytical Investigation of the Hydrolysis of Starch by Acid, *Journ. Amer. Chem. Soc.*, 1896, **18**, 869.

Rolfe and Faxton: The Exact Estimation of Total Carbohydrates in Acid Hydrolized Starch Products, *Journ. Amer. Chem. Soc.*, 1897, **19**, 698.

Hönig: Ueber Zusammensetzung und Untersuchung von Stärkesyrupen, *Ztschr. Unters. Nahr.-Genussm.*, 1902, **5**, 641.

# CHAPTER VII.

## Carbohydrates—Special Methods.

*(Continued.)*

### THE DETERMINATION OF STARCH.

### METHOD OF DIRECT ACID HYDROLYSIS.

This method is accurate for the determination of starch in such samples as contain no other substance insoluble in water and capable of yielding reducing sugar on heating with dilute acid. It is now well known that such interfering substances, especially the pentosans, are generally found in vegetable tissues, and that the results obtained by direct acid hydrolysis are usually too high. This is, however, the method which has been commonly used until recently and for comparisons with earlier work determinations by direct hydrolysis are still frequently required.

*Conversion of Starch to Dextrose.**

Weigh 2 to 5 grams and, if much fat is present, wash with four or five successive portions each 10 c.c. of ether; allow the ether to evaporate, and wash with 150 c.c. of 10 per cent. alcohol to remove soluble carbohydrates. Wash the residue into a 250 c.c. flask with 200 c.c. of water, add 20 c.c. of hydrochloric acid of 1.125 sp. gr., and heat in a boiling water bath with a reflux condenser for two and one-half hours. Cool to room temperature, nearly neutralize with sodium hydroxide, dilute to 250 c.c.; filter, and determine dextrose in a portion of the filtrate, using either Defren's method, already described, or Allihn's method, as follows:

*Allihn's Method for the Determination of Dextrose.*†

*Reagents.*—(1) 34.64 grams of crystallized copper sulphate dissolved in water and diluted to 500 c.c.

(2) 173 grams of sodium potassium tartrate and 125 grams of potassium hydroxide dissolved in water and diluted to 500 c.c.

* Sachsse: *Chem. Centrbl.*, 1877, 732; Bul. 46, Bur. Chem. U. S. Dept. Agriculture.

† *Journ. prakt. Chem.*, 1880, 22, 46; Bul. 46, *loc. cit.*

*Determination.*—Place 30 c.c. of the copper solution, 30 c.c. of the alkaline tartrate solution and 60 c.c. of water in a beaker or casserole and heat to boiling. To the boiling liquid add 25 c.c. of the dextrose solution, note the time at which the mixture begins to boil and continue the boiling for exactly two minutes. Filter at once and obtain the weight of copper in the precipitated cuprous oxide by any of the methods suggested in connection with Defren's process described in Chapter V, or as follows: Collect the cuprous oxide on an asbestos filter in a Gooch crucible; wash the precipitate (including any which may adhere to the beaker and which need not be transferred to the filter) thoroughly with hot water, transfer the asbestos and adhering oxide from the crucible to the beaker. Dissolve the oxide still remaining in the crucible by means of 1 to 2 c.c. of concentrated nitric acid, adding the acid from a pipette and receiving the solution in the beaker containing the asbestos and the main part of the precipitate. Rinse the crucible with a jet of water allowing the rinsings to flow into the beaker. Heat the contents of the beaker until all copper is in solution; filter, wash thoroughly, dilute the filtrate to 100 to 150 c.c., add one drop of concentrated sulphuric acid and determine copper by electrolysis. Find the corresponding weight of dextrose from Allihn's table.

*Notes.*—The conditions described must be observed carefully. The dextrose solution added to the copper reagent must be free from turbidity and only faintly acid. The rapid addition of this cold solution stops the boiling of the reagent, and it is well to have the heat so regulated that the mixture will boil again in about two minutes, then after exactly two minutes of actual boiling remove the flame and filter the solution at once. The cuprous oxide is very apt to run through the filter. To prevent this, after making the asbestos filter as usual, pour on it some very fine asbestos suspended in water, so as to form a tight layer on the top of the felt. On the assumption that the starch is quantitatively hydrolyzed to dextrose, the weight of the latter multiplied by 0.9 gives the corresponding weight of starch. For detailed studies of the hydrolysis of starch by acids, see the papers of Rolfe * and of Noyes.†

* *Journ. Amer. Chem. Soc.*, 1896, **18**, 869; 1897, **19**, 261; 1903, **25**, 1003, 1015
† *Ibid.*, 1904, **26**, 266.

ALLIHN'S TABLE FOR THE DETERMINATION OF DEXTROSE.

| Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper. | Milli-grams of Dex-trose. |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 6.1 | 67 | 34.3 | 124 | 63.1 | 181 | 92.6 | 238 | 122.8 |
| 11 | 6.6 | 68 | 34.8 | 125 | 63.7 | 182 | 93.1 | 239 | 123.4 |
| 12 | 7.1 | 69 | 35.3 | 126 | 64.2 | 183 | 93.7 | 240 | 123.9 |
| 13 | 7.6 | 70 | 35.8 | 127 | 64.7 | 184 | 94.2 | 241 | 124.4 |
| 14 | 8.1 | 71 | 36.3 | 128 | 65.2 | 185 | 94.7 | 242 | 125.0 |
| 15 | 8.6 | 72 | 36.8 | 129 | 65.7 | 186 | 95.2 | 243 | 125.5 |
| 16 | 9.0 | 73 | 37.3 | 130 | 66.2 | 187 | 95.7 | 244 | 126.0 |
| 17 | 9.5 | 74 | 37.8 | 131 | 66.7 | 188 | 96.3 | 245 | 126.6 |
| 18 | 10.0 | 75 | 38.3 | 132 | 67.2 | 189 | 96.8 | 246 | 127.1 |
| 19 | 10.5 | 76 | 38.8 | 133 | 67.7 | 190 | 97.3 | 247 | 127.6 |
| 20 | 11.0 | 77 | 39.3 | 134 | 68.2 | 191 | 97.8 | 248 | 128.1 |
| 21 | 11.5 | 78 | 39.8 | 135 | 68.8 | 192 | 98.4 | 249 | 128.7 |
| 22 | 12.0 | 79 | 40.3 | 136 | 69.3 | 193 | 98.9 | 250 | 129.2 |
| 23 | 12.5 | 80 | 40.8 | 137 | 69.8 | 194 | 99.4 | 251 | 129.7 |
| 24 | 13.0 | 81 | 41.3 | 138 | 70.3 | 195 | 100.0 | 252 | 130.3 |
| 25 | 13.5 | 82 | 41.8 | 139 | 70.8 | 196 | 100.5 | 253 | 130.8 |
| 26 | 14.0 | 83 | 42.3 | 140 | 71.3 | 197 | 101.0 | 254 | 131.4 |
| 27 | 14.5 | 84 | 42.8 | 141 | 71.8 | 198 | 101.5 | 255 | 131.9 |
| 28 | 15.0 | 85 | 43.4 | 142 | 72.3 | 199 | 102.0 | 256 | 132.4 |
| 29 | 15.5 | 86 | 43.9 | 143 | 72.9 | 200 | 102.6 | 257 | 133.0 |
| 30 | 16.0 | 87 | 44.4 | 144 | 73.4 | 201 | 103.1 | 258 | 133.5 |
| 31 | 16.5 | 88 | 44.9 | 145 | 73.9 | 202 | 103.7 | 259 | 134.1 |
| 32 | 17.0 | 89 | 45.4 | 146 | 74.4 | 203 | 104.2 | 260 | 134.6 |
| 33 | 17.5 | 90 | 45.9 | 147 | 74.9 | 204 | 104.7 | 261 | 135.1 |
| 34 | 18.0 | 91 | 46.4 | 148 | 75.5 | 205 | 105.3 | 262 | 135.7 |
| 35 | 18.5 | 92 | 46.9 | 149 | 76.0 | 206 | 105.8 | 263 | 136.2 |
| 36 | 18.9 | 93 | 47.4 | 150 | 76.5 | 207 | 106.3 | 264 | 136.8 |
| 37 | 19.4 | 94 | 47.9 | 151 | 77.0 | 208 | 106.8 | 265 | 137.3 |
| 38 | 19.9 | 95 | 48.4 | 152 | 77.5 | 209 | 107.4 | 266 | 137.8 |
| 39 | 20.4 | 96 | 48.9 | 153 | 78.1 | 210 | 107.9 | 267 | 138.4 |
| 40 | 20.9 | 97 | 49.4 | 154 | 78.6 | 211 | 108.4 | 268 | 138.9 |
| 41 | 21.4 | 98 | 49.9 | 155 | 79.1 | 212 | 109.0 | 269 | 139.5 |
| 42 | 21.9 | 99 | 50.4 | 156 | 79.6 | 213 | 109.5 | 270 | 140.0 |
| 43 | 22.4 | 100 | 50.9 | 157 | 80.1 | 214 | 110.0 | 271 | 140.6 |
| 44 | 22.9 | 101 | 51.4 | 158 | 80.7 | 215 | 110.6 | 272 | 141.1 |
| 45 | 23.4 | 102 | 51.9 | 159 | 81.2 | 216 | 111.1 | 273 | 141.7 |
| 46 | 23.9 | 103 | 52.4 | 160 | 81.7 | 217 | 111.6 | 274 | 142.2 |
| 47 | 24.4 | 104 | 52.9 | 161 | 82.2 | 218 | 112.1 | 275 | 142.8 |
| 48 | 24.9 | 105 | 53.5 | 162 | 82.7 | 219 | 112.7 | 276 | 143.3 |
| 49 | 25.4 | 106 | 54.0 | 163 | 83.3 | 220 | 113.2 | 277 | 143.9 |
| 50 | 25.9 | 107 | 54.5 | 164 | 83.8 | 221 | 113.7 | 278 | 144.4 |
| 51 | 26.4 | 108 | 55.0 | 165 | 84.3 | 222 | 114.3 | 279 | 145.0 |
| 52 | 26.9 | 109 | 55.5 | 166 | 84.8 | 223 | 114.8 | 280 | 145.5 |
| 53 | 27.4 | 110 | 56.0 | 167 | 85.3 | 224 | 115.3 | 281 | 146.1 |
| 54 | 27.9 | 111 | 56.5 | 168 | 85.9 | 225 | 115.9 | 282 | 146.6 |
| 55 | 28.4 | 112 | 57.0 | 169 | 86.4 | 226 | 116.4 | 283 | 147.2 |
| 56 | 28.8 | 113 | 57.5 | 170 | 86.9 | 227 | 116.9 | 284 | 147.7 |
| 57 | 29.3 | 114 | 58.0 | 171 | 87.4 | 228 | 117.4 | 285 | 148.3 |
| 58 | 29.8 | 115 | 58.6 | 172 | 87.9 | 229 | 118.0 | 286 | 148.8 |
| 59 | 30.3 | 116 | 59.1 | 173 | 88.5 | 230 | 118.5 | 287 | 149.4 |
| 60 | 30.8 | 117 | 59.6 | 174 | 89.0 | 231 | 119.0 | 288 | 149.9 |
| 61 | 31.3 | 118 | 60.1 | 175 | 89.5 | 232 | 119.6 | 299 | 150.5 |
| 62 | 31.8 | 119 | 60.6 | 176 | 90.0 | 233 | 120.1 | 290 | 151.0 |
| 63 | 32.3 | 120 | 61.1 | 177 | 90.5 | 234 | 120.7 | 291 | 151.6 |
| 64 | 32.8 | 121 | 61.6 | 178 | 91.1 | 235 | 121.2 | 292 | 152.1 |
| 65 | 33.3 | 122 | 62.1 | 179 | 91.6 | 236 | 121.7 | 293 | 152.7 |
| 66 | 33.8 | 123 | 62.6 | 180 | 92.1 | 237 | 122.3 | 294 | 153.2 |

ALLIHN'S TABLE FOR THE DETERMINATION OF DEXTROSE.—*Continued.*

| Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper. | Milli-grams of Dex-trose | Milli-grams of Copper. | Milli-grams of Dex-trose. | Milli-grams of Copper | Milli-grams of Dex-trose | Milli-grams of Copper. | Milli-grams of Dex-trose. |
|---|---|---|---|---|---|---|---|---|---|
| 295 | 153.8 | 329 | 172.5 | 363 | 191.7 | 397 | 211.2 | 431 | 231.0 |
| 296 | 154.3 | 330 | 173.1 | 364 | 192.3 | 398 | 211.7 | 432 | 231.6 |
| 297 | 154.9 | 331 | 173.7 | 365 | 192.9 | 399 | 212.3 | 433 | 232.2 |
| 298 | 155.4 | 332 | 174.2 | 366 | 193.4 | 400 | 212.9 | 434 | 232.8 |
| 299 | 156.0 | 333 | 174.8 | 367 | 194.0 | 401 | 213.5 | 435 | 233.4 |
| 300 | 156.5 | 334 | 175.3 | 368 | 194.6 | 402 | 214.1 | 436 | 233.9 |
| 301 | 157.1 | 335 | 175.9 | 369 | 195.1 | 403 | 214.6 | 437 | 234.5 |
| 302 | 157.6 | 336 | 176.5 | 370 | 195.7 | 404 | 215.2 | 438 | 235.1 |
| 303 | 158.2 | 337 | 177.0 | 371 | 196.3 | 405 | 215.8 | 439 | 235.7 |
| 304 | 158.7 | 338 | 177.6 | 372 | 196.8 | 406 | 216.4 | 440 | 236.3 |
| 305 | 159.3 | 339 | 178.1 | 373 | 197.4 | 407 | 217.0 | 441 | 236.9 |
| 306 | 159.8 | 340 | 178.7 | 374 | 198.0 | 408 | 217.5 | 442 | 237.5 |
| 307 | 160.4 | 341 | 179.3 | 375 | 198.6 | 409 | 218.1 | 443 | 238.1 |
| 308 | 160.9 | 342 | 179.8 | 376 | 199.1 | 410 | 218.7 | 444 | 238.7 |
| 309 | 161.5 | 343 | 180.4 | 377 | 199.7 | 411 | 219.3 | 445 | 239.3 |
| 310 | 162.0 | 344 | 180.9 | 378 | 200.3 | 412 | 219.9 | 446 | 239.8 |
| 311 | 162.6 | 345 | 181.5 | 379 | 200.8 | 413 | 220.4 | 447 | 240.4 |
| 312 | 163.1 | 346 | 182.1 | 380 | 201.4 | 414 | 221.0 | 448 | 241.0 |
| 313 | 163.7 | 347 | 182.6 | 381 | 202.0 | 415 | 221.6 | 449 | 241.6 |
| 314 | 164.2 | 348 | 183.2 | 382 | 202.5 | 416 | 222.2 | 450 | 242.2 |
| 315 | 164.8 | 349 | 183.7 | 383 | 203.1 | 417 | 222.8 | 451 | 242.8 |
| 316 | 165.3 | 350 | 184.3 | 384 | 203.7 | 418 | 223.3 | 452 | 243.4 |
| 317 | 165.9 | 351 | 184.9 | 385 | 204.3 | 419 | 223.9 | 453 | 244.0 |
| 318 | 166.4 | 352 | 185.4 | 386 | 204.8 | 420 | 224.5 | 454 | 244.6 |
| 319 | 167.0 | 353 | 186.0 | 387 | 205.4 | 421 | 225.1 | 455 | 245.2 |
| 320 | 167.5 | 354 | 186.6 | 388 | 206.0 | 422 | 225.7 | 456 | 245.7 |
| 321 | 168.1 | 355 | 187.2 | 389 | 206.5 | 423 | 226.3 | 457 | 246.3 |
| 322 | 168.6 | 356 | 187.7 | 390 | 207.1 | 424 | 226.9 | 458 | 246.9 |
| 323 | 169.2 | 357 | 188.3 | 391 | 207.7 | 425 | 227.5 | 459 | 247.5 |
| 324 | 169.7 | 358 | 188.9 | 392 | 208.3 | 426 | 228.0 | 460 | 248.1 |
| 325 | 170.3 | 359 | 189.4 | 393 | 208.8 | 427 | 228.6 | 461 | 248.7 |
| 326 | 170.9 | 360 | 190.0 | 394 | 209.4 | 428 | 229.2 | 462 | 249.3 |
| 327 | 171.4 | 361 | 190.6 | 395 | 210.0 | 429 | 229.8 | 463 | 249.9 |
| 328 | 172.0 | 362 | 191.1 | 396 | 210.6 | 430 | 230.4 | | |

According to several investigators,* the weight of dextrose should be multiplied by a higher factor (0.917 to 0.941) rather than 0.9, to find the corresponding weight of starch.

## METHOD OF DIGESTION WITH DIASTASE OR SALIVA.

If starch is gelatinized by boiling with water and then treated with malt diastase or saliva, it can be converted into maltose and dextrin and these separated by filtration from the insoluble residue

* Salomon : *Ztschr. anal. Chem.*, 1883, **22**, 593. Soxhlet : *Wochenschr. für Brauer.*, 1885, 193 ; Vaubel, II., 455. Sostegni : *Chem. Centrbl.*, 1887, **58**, 896. Lintner and Düll : *Ztschr. angew. Chem.*, 1891, 537. Ost : *Chem. Ztg.*, 1895, **19**, 1502. Rössing : *Ztschr. öffentl. Chem.*, 1904, **10**, 61 ; Abs. *Journ. Chem. Soc.*, 1904, **86**, ii, 298. Noyes : *Journ. Amer. Chem. Soc.*, 1904, **26**, 280.

containing the pentosans and other substances which cause the results by the preceding method to be too high.

*Determination.* — Extract 2.5 to 5 grams of sample with five successive portions each 10 c.c. of ether, decanting the washings through a hardened filter; wash with 150 c.c. of 10 per cent. alcohol;* transfer the residue to a beaker with 50 to 100 c.c. of water; heat gradually to boiling, stirring constantly to prevent bumping or the formation of lumps. Cool to 55° for diastase, or to 38° for saliva; add the solution containing the enzyme and keep the mixture within two degrees of the stated temperature until a drop, removed and tested on a porcelain plate, no longer shows a reaction for starch on mixing with a drop of dilute solution of iodine in aqueous potassium iodide. Now heat the solution again to boiling in order to gelatinize any starch granules which may remain; test the solution, and if starch is found, cool to the proper temperature; add more of the enzyme and digest as before. Continue this treatment until the solution gives no starch reaction after boiling, or until a careful microscopic examination shows that the insoluble residue is entirely free from starch. Dilute to 250 c.c., mix thoroughly and pour on a dry fluted filter. Transfer 150 c.c. of the filtrate to a 250 c.c. flask; add 15 c.c. of hydrochloric acid of 1.125 sp. gr., attach the flask to a reflux condenser and heat in a boiling water-bath for two and one-half hours, or boil gently on a hot plate or sand-bath for 35 to 45 minutes. Complete the determination as described in the preceding method.

The determination should be carried through without interruption. If this is impossible, care must be taken to avoid alcoholic or lactic fermentation. After the digestion with the enzyme is finished, but not before, salicylic acid may be added as a preservative. It has been recommended that a trace of fluoride be added at the start to retard lactic fermentation while the digestion with the enzyme is taking place.

When only a few determinations are to be made, freshly collected saliva can conveniently be used, as this is free from carbohydrate. If commercial diastase or an infusion of malt † is used,

* This extraction can often be omitted, since for many purposes it is unnecessary to distinguish between starch and soluble carbohydrates.

† An active malt infusion can be prepared by digesting 10 grams of fresh, finely-ground malt, over night at room temperature, with 200 c.c. of water or 10 per cent. alcohol.

the amount added must be noted and a correction applied for the carbohydrate thus introduced. This is found by heating a quantity of the diastate or infusion with acid and determining the resulting dextrose as in the starch determination.

### Comparison of Results.

The diastase method, carefully carried out, is believed to yield practically correct results. As already explained, the results obtained by direct acid hydrolysis are usually higher owing to the presence of other substances which yield reducing sugars. A comparison of the results of the two methods is of considerable interest, both because many of the recorded determinations of starch were made by the acid method and because the determination of copper-reducing substance obtained by direct hydrolysis is sometimes used as a means of detecting adulterants in spices. The table below shows the results of comparison of the two methods on a variety of substances. Many of the results of Winton and associates are averaged from the comparative examination of several samples. The other results were obtained by the writer, only one sample of each kind being examined.

*Results by Diastase and by Direct Acid Hydrolysis.*

| Substance. | Starch Indicated by | | Substance | Starch Indicated by | |
|---|---|---|---|---|---|
| | Diastase Method Per Cent. | Acid Method Per Cent. | | Diastase Method Per Cent. | Acid Method Per Cent. |
| Air-dry starch | 82.49 | 82.30 | White pepper* | 56.47 | 59.17 |
| Wheat flour | 66.55 | 68.35 | Long pepper* | 39.55 | 42.88 |
| Oatmeal | 56.23 | 59.01 | Black pepper* | 34.15 | 38.63 |
| Graham flour | 55.32 | 58.63 | Mace* | 27.87 | 31.73 |
| Wheat bran | 20.97 | 38.82 | Nutmeg* | 23.72 | 25.56 |
| Linseed meal* | 14.06 | 21.15 | Allspice* | 3.04 | 18.03 |
| Cocoa nibs* | 8.07 | 11.16 | Cloves* | 2.74 | 8.99 |
| Wheat straw | 4.39 | 22.69 | Pepper shells* | 2.30 | 11.43 |
| Cocoa shells* | 4.14 | 18.03 | Cayenne* | 1.01 | 8.47 |
| Buckwheat hulls* | 1.46 | 20.51 | Walnut shells* | 1.01 | 19.30 |
| Corn stover | 0.96 | 20.13 | Almond shells* | 0.84 | 22.72 |

### Determination of Starch in Meat Products.

Starchy materials are sometimes added as "fillers" to sausages and other forms of chopped meat. This adulteration is easily de-

* Results by Winton and associates, compiled from Leach's Food Inspection and Analysis.

tected by the iodine reaction which, however, must be carefully interpreted since a small amount of starch may legitimately be present from the spices added in the manufacture. The quantitative determination is complicated by the fact that meat appears to contain some substance which interferes with the separation of the cuprous oxide reduced in applying the usual method. Advantage is therefore taken of the insolubility of starch in alcoholic, and its solubility in aqueous potassium hydroxide. The method of Mayrhofer as modified by Bigelow and adopted by the Association of Official Agricultural Chemists is as follows:*

Treat from 10 to 20 grams of the sample under examination (depending upon the amount of starch indicated by the iodine reaction) in a porcelain dish or casserole with 50 c.c. of an 8 per cent. solution of potassium hydroxide and heat the mixture on the water-bath until the meat is entirely dissolved. Add an equal volume of 95 per cent. alcohol, mix thoroughly, filter the mixture through an asbestos filter and wash twice with a hot 4 per cent. solution of potassium hydroxide in 50 per cent. alcohol. Then wash with 50 per cent. alcohol until a small portion of the filtrate does not become turbid upon the addition of acid. Return the precipitate and filter to the original vessel and dissolve the precipitate with the aid of heat in 60 c.c. of a normal solution of potassium hydroxide. In the case of sausage with a high starch content a somewhat larger volume of alkali may be required. Acidify the filtrate strongly with acetic acid, dilute to a definite volume, mix thoroughly by shaking, filter through a fluted paper, and precipitate the starch from an aliquot part of the filtrate by means of an equal volume of 95 per cent. alcohol. Transfer the precipitate to a weighed filter, wash thoroughly with 50 per cent. alcohol, with absolute alcohol, and finally with ether, dry to a constant weight at the temperature of boiling water and weigh.

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## SEPARATION OF CARBOHYDRATES IN CEREAL PRODUCTS.

### DETERMINATION OF REDUCING SUGARS, SUCROSE, DEXTRIN, STARCH, PENTOSANS AND CELLULOSE.

The following scheme * provides for each of the substances or groups mentioned and avoids the danger (inherent in the usual plan of making a number of independent determinations) of including the same substance as a constituent of more than one group.

Free the sample from fat by washing with ether.

Extract with boiling alcohol.

*Solution A.*— Evaporate the alcohol, dilute with water and determine the reducing power of portions of the solution before and after hydrolysis. Calculate the *reducing sugar* and *sucrose*.

*Residue A.* — Extract with cold water.

*Solution B.* — Hydrolyze a portion and determine the resulting dextrose, calculate *dextrin* and *soluble starch*. In another portion, precipitate soluble starch by barium hydroxide,† filter and determine *dextrin* in the filtrate.

*Residue B.* — Boil with water and treat with malt extract or saliva, filter and wash thoroughly.

*Solution C.* — Hydrolyze, determine resulting dextrose and calculate *starch*.

*Residue C.* — Boil with 2 per cent. hydrochloric or sulphuric acid until the maximum reducing power of the solution is reached.‡

*Solution D.* — Determine reducing power in the same manner as for dextrose. Calculate as xylose, the reducing power of which is 1.03 times that of dextrose. § From the pentose thus found cal-

* Based on the following papers: Stone; *Journ. Amer. Chem. Soc.*, 1897, **19**, 183. Sherman; *Ibid.*, 1897, **19**, 291. Browne and Beistle; *Ibid.*, 1901, **23**, 229.

† Asboth: *Chem. Ztg.*, 1889, **13**, 591.

‡ For sulphuric acid this was found to be 4 to 6 hours. Stone prefers hydrochloric acid and states that the reaction is nearly complete in 1 hour.

§ Stone: *Amer. Chem. Journ.*, 1891, **13**, 82. Since the reducing power of arabinose does not differ greatly from that of xylose, this calculation would still be nearly correct in case both pentoses were present.

culate the percentage of *pentosan*. In the case of wheat it has been found * that the material ("hemicellulose") dissolved and hydrolyzed at this point is entirely pentosan. The same is probably true of the other cereals. The pentosan thus dissolved and hydrolyzed does not include necessarily the entire furfurol-yielding substance of the cereal.

*Residue D.*—Boil for 30 minutes with 1 per cent. sodium hydroxide, filter and wash, press out most of the water and expose the moist fiber to chlorine gas for one hour. Wash with water, boil with a solution containing 2 per cent. sodium sulphite and 0.2 per cent. sodium hydroxide; filter, wash with warm water until the washings are neutral and colorless, then wash with strong alcohol, dry and weigh. Deduct the ash which the residue contains and calculate the organic matter as *cellulose*. †

DETERMINATION OF MALTOSE, DEXTRIN AND STARCH IN MALTED CEREAL.

The absence of any considerable amount of dextrose or invert-sugar must be shown by stirring some of the sample with about ten times its weight of water and testing the filtered extract by means of phenylhydrazine or Barfoed's solution as described in Chapter V. If no monosaccharide is present the percentages of maltose, dextrin and starch can be estimated as follows:

Mix 5 grams of sample with 125 c.c. of cold water‡ in a 250 c.c. flask; allow to stand at room temperature for one hour, shaking frequently; fill to the mark, shake and filter through dry paper. Determine reducing power of one or more 25 c.c. portions of this filtrate by either Defren's or Allihn's method and calculate the amount of maltose.§ Measure 50 c.c. of the same filtrate into a 100 c.c. flask, add 5 c.c. of hydrochloric acid of 1.125 sp. gr. and hydrolyze as in the determination of starch. Determine the resulting dextrose, deduct the amount due to maltose and estimate

* *Journ. Amer. Chem. Soc.*, 1897, **19**, 294.

† This is the method of Cross and Bevan. For a comparison of this with othe methods see *Journ. Amer. Chem. Soc.*, 1897, **19**, 304.

‡ If the sample contains an active enzyme some of the carbohydrate may be change during this treatment with water. To prevent this a very dilute alkali solution, cor taining 0.02 per cent. potassium hydroxide or an equivalent amount of sodium or am monium hydroxide, may be used. Ling and Rendle: *Journ. Inst. Brewing*, 1904, **1(** 238; Abs. *Journ. Chem. Soc.*, 1904, **86**, ii., 507.

§ If Allihn's method is used, assume the reducing power of maltose to be 0.6 that of dextrose.

the remainder as due to dextrin. Soluble starch if present would be counted as dextrin in this analysis.

Treat another portion of the original sample as described under the determination of starch, but without extracting the soluble carbohydrates. From the dextrose found, subtract that yielded by maltose and dextrin and estimate the remainder as derived from starch.

The results require a slight correction on account of the presence of the insoluble residue when the solution is diluted to volume in the graduated flask. Although some details of the method are open to criticism, it gives results sufficiently exact for the purpose for which it is mainly used, which is to show whether the starch of the cereal has been largely changed to soluble products.

The same plan may be used in the examination of cereal foods prepared by parching or in other ways, provided only one reducing sugar is present in appreciable quantity. The amount of soluble carbohydrate in such preparations is usually too small for satisfactory determination by means of the polariscope.

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## CHAPTER VIII.

### Acids.

Free organic acids when dissolved in water are dissociated to a small extent and can usually be determined by direct titration, using as standard alkali a solution of a strong base and as indicator a substance of very weak acid properties such as phenolphthalein. In cases where direct titration is not available, the determination of acidity can be made by an indirect method, such as allowing the substance to act upon a carbonate and collecting the carbon dioxide set free, or upon potassium iodate in the presence of an excess of iodide when each carboxyl hydrogen entering into reaction results in the liberation of one atom of iodine. The iodine thus liberated is determined by titration with standard thiosulphate. These and other methods for the determination of the carboxyl group are fully discussed by Meyer and Tingle.* Alkaline salts of organic acids are usually converted into carbonates by ignition, so that the determination of carbonate in the ash of a neutral or neutralized mixture often indicates the amount of organic acid present as salt before ignition.

#### ACETIC ACID AND ACETATES.

Pure acetic acid is a colorless liquid of 1.056 specific gravity, miscible in all proportions with water, alcohol, and ether. When nearly anhydrous it solidifies at about 16° — hence the term "glacial" as applied to very strong acetic acid. The pure acid boils without decomposition at 119°. From dilute aqueous solutions it distils readily with steam at the temperature of boiling water. The principal source of commercial acetic acid is the crude acetate of lime made by neutralizing the acid obtained in the dry distillation of wood. For the determination of the volatile acid obtainable from commercial acetate the following method can be used.

#### DETERMINATION OF ACETIC ACID IN CALCIUM ACETATE.

Arrange a round-bottomed flask of 300 c.c. capacity in such a way that it can be inclined at an angle of about 60° from the per-

* Determination of Radicles in Carbon Compounds.

pendicular and connected with a nearly vertical condenser, while another tube passing through the stopper of the flask provides for the continuous introduction of water, drop by drop, during the distillation. The flow of water can be controlled by means of a screw pinchcock or a small dropping funnel.

Weigh 2 grams of the ground sample,* transfer to the flask and add 15 c.c. of 50 per cent. phosphoric acid and 25 c.c. of water, being sure that the water washes down any of the sample or acid which may have adhered to the neck of the flask. Connect the flask with the condenser and distil, collecting the distillate in a receiver containing 50 c.c. of water to prevent loss due to evaporation of acetic acid. During the distillation keep the volume of liquid nearly constant at 40 c.c. by admitting water free from carbon dioxide, in such a way that the drops fall upon the inner surface of the neck of the flask and not directly into the boiling liquid. Continue the distillation until the distillate is no longer acid. This usually requires about one and one-half hours. Titrate the distillate with freshly standardized sodium hydroxide solution using phenolphthalein as indicator. Calculate the total acidity as percentage of acetic acid in the sample.

*Notes.* — It has been found that small amounts of phosphoric acid are frequently carried over mechanically if the acetic acid is removed by a current of steam or if the distillation is conducted in an upright flask, especially when drops of water are allowed to fall directly into the boiling acid mixture. The directions for arrangement of apparatus are intended to avoid this source of error.

The phosphoric acid used must not contain nitric or any other volatile acid. The large excess recommended dissolves the calcium phosphate formed and thus prevents bumping. Oxalic may be substituted for phosphoric acid, and the calcium oxalate removed by filtration before distilling. This method, however, is longer and no more accurate than the phosphoric acid method as described. Sulphuric acid cannot be used, as it would be partially reduced to sulphurous acid by the tarry matter present in the crude acetate.

The distillate obtained as described should contain only a minute amount of carbonic acid. It is frequently recommended that the distillate be caught in standard alkali nearly sufficient to neutralize

* In sampling, grinding, and weighing portions for analysis, special care must be taken to avoid changes in moisture content.

all the volatile acid expected. In this case the distillate would contain all carbonic acid liberated in the distillation. The distillate containing some free acetic acid should therefore be boiled under a reflux condenser to expel carbonic acid before making the final titration. Errors may also be caused by variation in the amount of carbonate in the alkali used for titration. Hence this must be freshly standardized, using phenolphthalein as indicator, under the same conditions of temperature and removal of carbon dioxide as exist in the titration of the distillate.

The volatile organic acid, though calculated as acetic, always contains some formic, propionic, and butyric acids. In addition to these Scheuer found small amounts of valerianic, caproic, heptylic, caprylic, and nonylic acids. In view of the danger of phosphoric acid being carried over, it is advisable, after titrating the distillate, to add nitric acid, evaporate to 25 c.c. and test for phosphoric acid by adding ammonium nitrate and molybdate solution.

The distillation method has now almost entirely replaced the indirect methods formerly used. There is, however, no general agreement as to the details of manipulation. A full description of the method as used in the laboratory of the General Chemical Company is given by Grosvenor.

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## Separation of Acetic Acid from Its Homologues.

It was explained, in connection with the determination of alcohols, that methyl alcohol is oxidized to carbon dioxide and water by heating with sulphuric acid and potassium dichromate while ethyl alcohol is oxidized only to acetic acid. In the same way formic acid can be determined by oxidation with the dichromate mixture, acetic acid, if present, being unaffected by the treatment. The methods of Macnair * and Freyer † are based on this principle.

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* *Chem. News*, 1887, **55**, 229.

† *Chem. Ztg.*, 1895, **19**, 1184.

According to Leys,* formic acid is also quantitatively oxidized by mercuric acetate. The reaction takes place readily on heating, even in very dilute solution, and is said to yield accurate results which are not affected by the presence of acetic acid, alcohol, or aldehyde.

Aside from the determination of formic acid by oxidation, the analysis of a mixture of closely related homologues is very difficult. Haberland proposed † a method for the separation of formic, acetic, propionic, and butyric acids in crude wood vinegar. This method involved the precipitation of propionic acid as basic lead propionate insoluble in water, and of formic acid as zinc formate insoluble in alcohol, after which the acetic and butyric acids were separated either by the difference in the solubilities of the silver salts or by fractional distillation of the amyl esters. According to Schütz,‡ however, these separations are far from quantitative.

Wechsler§ separated these acids qualitatively by the method of partial distillation, in which the mixture of acids is treated with an amount of alkali insufficient for complete neutralization, and then distilled. Under these conditions the higher homologues, although less volatile, are concentrated in the distillate, the lower and stronger acids remaining in combination with the base. Scheuer‖ applied the same method quantitatively with good results but found it too long for practical purposes.

## VINEGAR.

A thorough examination of vinegar should include determinations of specific gravity, total solids or extract, total and soluble ash, alkalinity and phosphoric acid of the ash, total and volatile acidity, and alcohol. Free mineral acids and oxalic acid should be determined if present and tests should be made for preservatives, poisonous metals, foreign pungent materials, and foreign colors.

Methods for all of these determinations have been adopted by the Association of Official Agricultural Chemists. The following directions for the more important determinations are in accordance with the official methods.

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* *Bull. Soc. Chim.*, 1898 [3], 19, 472; *Chem. News*, 1898, 78, 245. See also Sparre: *Ztschr. anal. Chem.*, 1900, 39, 105.

† *Ztschr. anal. Chem.*, 1899, 38, 217.

‡ *Ztschr. anal. Chem.*, 1900, 39, 17.

§ *Monatsh. Chem.*, 1893, 14, 462.

‖ Ueber die Trennung und Bestimmung flüchtiger Fettsauren. Dissertation, Munich, 1902.

## Solids and Ash.

### *Total Solids or Extract.*

Evaporate 10 c.c. nearly to dryness in a weighed platinum dish of 50 mm. diameter on a steam bath, dry for two and one-half hours in a boiling water oven, cool thoroughly in a desiccator and then weigh quickly.

### *Total Ash.*

Char the extract thoroughly at a low red heat, leach with water, burn the insoluble residue to whiteness, add the water solution, evaporate and heat to low redness, cool in a desiccator and weigh.

### *Solubility and Alkalinity of Ash.**

Evaporate 25 c.c. to dryness, burn, cool and weigh; extract the ash repeatedly on an ash-free filter; dry and ignite the filter and residue, and weigh as insoluble ash. Titrate the filtrate using methyl orange as indicator, and calculate the number of c.c. of tenth-normal acid which would be required to neutralize the corresponding filtrate from 100 c.c. of the vinegar.

## Total and Volatile Acids.

### *Total Acidity.*

Dilute 10 c.c. in a beaker until the solution appears very light-colored against a white background, add phenolphthalein and titrate with standard sodium hydroxide. If only the total acidity is determined the result is expressed as acetic acid.

### *Volatile Acids.*

Heat 15 c.c. of the vinegar to boiling in a flask, adding a little tannin to check foaming, if necessary; lower the flame and distil with steam until the distillate no longer contains acid. Titrate the distillate with standard sodium hydroxide and calculate as acetic acid.

The difference between the total acidity and that due to volatile acids gives a measure of the fixed acids and, in the case of cider vinegar, is calculated as malic acid.

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* Smith's method modified by Frear: *Journ. Amer. Chem. Soc.*, 1898, **20**, 5; Bul. **65**, Bur. Chem., U. S. Dept. Agriculture.

## FREE MINERAL ACIDS.

### *Detection by Methyl Violet.*

Dilute 5 c.c. of the vinegar with 5 to 10 c.c. water to reduce the acidity to about 2 per cent. of acetic acid, add four or five drops of an aqueous solution of methyl violet (one part of "methyl violet 2B" — No. 56 of Bayer Farbenfabrik, Elberfeld — in 10,000 parts of water). Mineral acids change the blue violet color to a blue green or green.

### *Determination — Hehner's Method.*

To a weighed quantity of the sample, add a measured amount (more than sufficient to neutralize all mineral acid present) of tenth-normal alkali; evaporate to dryness, incinerate and titrate the ash with tenth-normal acid using methyl orange as indicator. The difference between the volume of alkali added and that of acid required, represents the equivalent of the free mineral acid in the sample.

## DETERMINATION OF SOURCE.*

The principal vinegar of the United States is cider vinegar, although considerable quantities of malt vinegar are also used. The substitutes are made mainly of low-grade wine products, sugar house or glucose wastes, and wood vinegar from acetate of lime. Cheap apple jelly is sometimes added to the substitutes to give them the color, flavor, and body of cider vinegar.

Cider vinegar contains from 1.2 to 8 per cent. of solids, average about 2.5 per cent.; malt vinegar 1.75 to 6, average about 3 per cent.; spirit vinegar rarely over 0.75 per cent., average about 0.30 per cent.

The total ash in spirit and wood vinegars rarely exceeds 0.1 per cent. In fruit and malt vinegars it rarely falls below 0.2, cider vinegar averaging about 0.35 per cent.

The alkalinity of the ash, expressed as above, is, according to Frear, for cider vinegar 26 to 65, average 39; for malt vinegar 5.5; for spirit vinegar 1.1.

The flame reaction of the solids is said to be always that of potash in the case of cider vinegar, while spirit, sugar, and glucose vinegars and any which have been artificially colored show the sodium flame (Davenport-Frear).

* Cf. Frear: Bul. 65, *loc. cit.*

The optical activity of vinegar often indicates the source. Pure cider vinegar, after clarification with basic lead acetate, is lævorotatory, a 200 mm. tube giving usually a reading of $-0.5^{\circ}$ to $-1.4^{\circ}$ on the Ventzke scale. According to Browne, levulose is the only sugar present in properly fermented cider vinegar, the sucrose and dextrose having both disappeared in the alcoholic fermentation. Wine vinegar is also slightly lævorotatory. Vinegar from sugar house wastes is dextrorotatory before, and lævorotatory after, hydrolysis. Glucose vinegar shows dextrorotation both before and after hydrolysis. Artificial vinegars can, of course, be made lævorotatory by the addition of apple pomace.

## Official Standards.

The definitions and standards established by the Secretary of Agriculture under authority of Congress are in part as follows: *

*Vinegar, cider vinegar*, or *apple vinegar* is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples, is lævorotatory, and contains not less than 4 grams of acetic acid, not less than 1.6 grams of apple solids, and not less than 0.25 gram of apple ash in 100 c.c. The water-soluble ash from 100 c.c. of the vinegar neutralizes not less than 30 c.c. of tenth-normal acid and contains not less than 0.010 gram of phosphoric anhydride.

Wine or grape vinegar, malt vinegar, sugar vinegar, glucose vinegar, and spirit or grain vinegar are also defined with special reference to the materials from which they are prepared and must all contain at least the amount of acetic acid prescribed under cider vinegar.

## References.

Allen: Commercial Organic Analysis, Volume I.

Leach: Food Inspection and Analysis.

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The Effects of Fermentation upon the Composition of Cider and Vinegar, *Journ. Amer. Chem. Soc.*, 1903, **25**, 16.

Frear: Vinegar, Bul. 65, Bur. Chem., U. S. Dept. Agriculture.

Leach and Lythgoe: Cider Vinegar and Suggested Standards of Purity, *Journ. Amer. Chem. Soc.*, 1904, **26**, 375.

* U. S. Dept. Agriculture, Office of the Secretary, Circular No. 13.

Van Slyke: A Study of the Chemistry of Home-made Cider Vinegar, Bul. 258, New York Agricultural Experiment Station, 1904.

FATTY ACIDS.

The fatty acids are all monobasic * and those of the common fats all contain an even number of carbon atoms in the molecule. Fatty acids containing odd numbers of carbon atoms are not widely distributed in nature, being usually characteristic of some particular fat or small group of fats. The following statement of analytical properties is based mainly upon the data given by Lewkowitsch.†

ACIDS OF THE SERIES $C_nH_{2n}O_2$.

*Butyric acid*, $C_4H_8O$, occurs ‡ in butter and in very small quantity in a few other fats. It is a mobile liquid, mixing in all proportions with water, alcohol, and ether. It boils without decomposition at 162° and is readily volatile with steam. Specific gravity at 20°, 0.959.

*Caproic acid*, $C_6H_{12}O_2$, occurs in butter and cocoanut fat. It is a rather oily liquid of unpleasant odor, not miscible with water but somewhat soluble in it and volatile with steam. Boiling point about 200°. Specific gravity at 20°, 0.924.

*Caprylic acid*, $C_8H_{16}O_2$, occurs in cocoanut oil, butter, and human fat. In the cold it crystallizes in leaflets which melt at 16.5°. It boils at 237° and is volatile with steam. One part dissolves in 400 parts of boiling water from which it separates almost completely on cooling. It is readily soluble in alcohol and ether. Specific gravity at 20°, 0.910.

*Capric acid*, $C_{10}H_{20}O_2$, has been found chiefly in cocoanut oil, butter, and the fat of the spice bush, *Lindera benzoin*. It melts at 31.3°, boils at 270°, and distils with steam. Soluble in about 1,000 parts of boiling water; almost insoluble in cold water; soluble in alcohol and ether.

*Lauric acid*, $C_{12}H_{24}O_2$, occurs abundantly in the fat of the seeds of *Lindera benzoin*, and in smaller proportions in butter, cocoanut fat, palm oil, laurel oil, and the nut oil of the California bay tree. Lauric acid melts at 43.6° and cannot be distilled at atmospheric pressure without decomposition. It is practically insoluble in cold

* For a possible exception see Gietel and Want: *Journ. prakt. Chem.*, 1900, 61, 151.

† Oils, Fats, and Waxes (Third Edition).

‡ These statements refer to the occurrence of the acids as esters rather than in the free state.

water, slightly soluble in boiling water and appreciably volatile with steam. Specific gravity at 20°, 0.883.

*Myristic acid*, $C_{14}H_{28}O_2$, is found in nutmeg butter, dika fat, butter, cocoanut oil, lard, and many other fats, as well as in spermaceti and wool wax. It crystallizes in leaflets which melt at 53.8°. Myristic acid is insoluble in water and only very slightly volatile with steam. It is not readily soluble in cold alcohol.

*Palmitic acid*, $C_{16}H_{32}O_2$, occurs in nearly all solid fats and non-drying oils as well as in several waxes including spermaceti and beeswax. It melts at 62.6° and solidifies on cooling to a scaly crystalline mass. It is insoluble in water and not readily soluble in cold 95 per cent. alcohol, but dissolves in about 10 parts of cold absolute alcohol.

*Stearic acid*, $C_{18}H_{36}O_2$, is found in most fats and occurs most abundantly in those having high melting points. It crystallizes in leaflets which melt at 69.3°. It is less soluble in alcohol than palmitic acid, requiring about 40 parts of absolute alcohol in the cold. It dissolves readily in ether, benzol, carbon bisulphide, or hot alcohol.

*Arachidic acid*, $C_{20}H_{40}O_2$, occurs in arachis (peanut) oil and has been obtained in very small quantities from several other fats. Arachidic acid is distinguished from all its lower homologues by its insolubility in cold alcohol. It dissolves freely in hot alcohol but on cooling separates almost completely as needles or crystalline scales, which melt at 77° to 78°.

*Behenic acid*, $C_{22}H_{44}O_2$, *lignoceric acid*, $C_{24}H_{48}O_2$, and an isomer of the latter, *carnaübic acid*, occur respectively in oil of ben, arachis oil, and carnaüba wax.

*Cerotic acid*, $C_{26}H_{52}O_2$, and *melissic acid*, $C_{30}H_{60}O_2$, are found in the free state in beeswax.

The change in properties of these acids, as the molecular weight increases, is noticeably regular. The melting- and boiling-points rise while the specific gravities and solubilities decrease. In general the properties of the glycerides and other esters vary in the same way as those of the free acids.

With the exception of caproic, all of the acids of this series, occurring in natural fats, are believed to be of the "normal" (straight chain) structure.

ACIDS OF THE SERIES $C_nH_{2n-2}O_2$.

[illegible] Each molecule contains one ethy-

lene linkage or double bond and is, therefore, capable of taking up by direct addition two atoms of halogen or one molecule of hydrobromic acid to form a saturated compound. Careful oxidation in the presence of moisture results in the formation of the corresponding dihydroxy-compounds. The unsaturated acids have as a rule, lower melting points than the saturated acids containing the same number of carbon atoms, and are therefore found more largely in oils and soft fats. Only the more important members of the series will be considered here.

*Hypogæic acid*, $C_{16}H_{30}O_2$, obtained from arachis (peanut) oil, melts at 33°, but is converted by the action of nitrous acid into the isomeric gaidic acid which melts at 39°. *Physetoleic acid*, isomeric with hypogæic, is obtained from sperm oil and seal oil. It melts at 30° and is not changed by nitrous acid.

*Oleic acid*, $C_{18}H_{34}O_2$, occurs in nearly all fats and fatty oils. It is an oily liquid which solidifies at 4° and melts at 14°. Nitrous acid converts oleic acid into the isomeric elaidic acid which is a crystalline solid melting at 44.5° (Lewkowitsch).

*Erucic acid*, $C_{22}H_{42}O_2$, found in rape seed and mustard seed oils melts at 33° to 34°. By the action of nitrous or sulphurous acid it can be converted into brassidic acid, melting at 65°. This change, however, takes place less readily than the corresponding conversion of oleic acid into elaidic acid.

The gradual change in properties with increasing molecular weight, noticed in the saturated acids, is not apparent in this series doubtless because the known acids of the series differ as regards the position of the double bond and are therefore not strictly similar in constitution. The property of changing to solid isomers under the influence of nitrous acid is characteristic of the acids of this series and furnishes a means of testing for them in fatty oils. Any of the latter which contain large amounts of these acids become solid or semi-solid on treatment with nitrous acid. This is known as the *elaidin test* and is sometimes useful in the preliminary examination of oils, but has been shown to be less reliable than was formerly supposed.

An important characteristic of the unsaturated acids is the solubility of the lead soaps in ether. Muter's method for the separation of saturated and unsaturated fatty acids, is to precipitate the mixture of acids as lead soaps and wash with ether. While this method is not strictly quantitative, it is probably the best which has been devised for the purpose.

### ACIDS OF THE SERIES $C_nH_{2n-4}O_2$.

The acids of this series unite with four atoms of bromine or iodine and absorb oxygen on exposure to the air.

*Linoleic acid*, $C_{18}H_{32}O_2$, is the only important member of the series. It is widely distributed occurring most abundantly in the "drying oils," so called because they are oxidized to solids on exposure to air. While especially characteristic of the drying and semi-drying oils, linoleic acid is also found in nondrying oils and in small quantity in some of the solid fats. It absorbs oxygen from the air so readily as to interfere seriously with ordinary methods of purification and examination. Its physical properties are therefore not well known but it has been shown to have a higher specific gravity than oleic acid and a much lower melting point since it does not solidify at $-18°$. Linoleic acid is not changed by nitrous acid. Lead linoleate is soluble in ether.

### ACIDS OF THE SERIES $C_nH_{2n-6}O_2$.

*Linolenic acid*, $C_{18}H_{30}O_2$, is the only member of this series which has been obtained in the free state. It is a colorless oil which absorbs oxygen very rapidly from the air, at the same time becoming dark brown. The purest preparations of the acid which have been described absorbed 242 to 245 per cent. of iodine. The pure acid should absorb six atoms or 274 per cent. of iodine. Linolenic acid has not been solidified. Its specific gravity is greater than that of linoleic acid. The lead soap is soluble in ether. Linolenic acid occurs in considerable quantity in linseed oil. By oxidizing linseed oil acids with alkaline permanganate Hazura obtained two acids of the formula $C_{18}H_{30}(OH)_6O_2$, linusic and isolinusic acids, and hence inferred the presence of an isolinolenic acid in linseed oil. Jecoric acid, isomeric with linolenic acid, is reported by Fahrion * as existing in sardine oil. Since the oils of fish and other marine animals do not dry like the vegetable oils having the same iodine absorbing power, it is probable that the highly unsaturated acids of the former oils are in general isomeric, rather than identical, with linolenic acid. Some of the fish oils are thought to contain acids of the series $C_nH_{2n-6}O_2$

### SATURATED HYDROXY-ACIDS.

When any of the unsaturated acids occurring in vegetable oils is carefully oxidized in alkaline solution by means of potassium

* *Chem. Ztg.*, 1893, 17, 521.

permanganate, hydroxyl is added until the molecule becomes saturated. Thus oleic acid yields dihydroxystearic acid, $C_{18}H_{34}(OH)_2O_2$, linoleic acid yields sativic acid, $C_{18}H_{32}(OH)_4O_2$, and linolenic acid yields linusic acid, $C_{18}H_{30}(OH)_6O_2$. Similar changes in the unsaturated acids may take place when their glycerides are exposed to the air. Small amounts of saturated hydroxy-acids are, therefore, likely to be present in fatty oils which have been kept for some time in partially filled bottles or otherwise exposed to atmospheric oxidation. Only two acids of this series have as yet been found in nature. Juillard* found about 1 per cent. of dihydroxystearic acid in castor oil and Browne† the same quantity in butter fat. A dihydroxy-acid of the formula, $C_{30}H_{60}O_4$, is said to occur in wool wax‡ and is called lanocerinic or lanoceric acid.

### Hydroxy-Acid of the Series $C_nH_{2n-2}O_3$.

*Ricinoleic acid*, $C_{18}H_{34}O_3$, the only important member of this series, occurs in large quantity in castor oil. It is a very viscous liquid of much higher specific gravity than oleic or linoleic acid. According to Juillard§ the pure acid melts at 4°–5°, and is miscible with alcohol and ether in all proportions. Ricinoleic acid is dextrorotatory. The position of the asymetric carbon atom, as well as of the double bond, is shown by the following formula‖

$$C_6H_{13} \cdot CH(OH) \cdot CH_2 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH.$$

Ricinoleic resembles oleic acid in its chemical reactions. It absorbs two atoms of bromine or iodine. By treatment with permanganate in alkaline solution two hydroxyl radicals are added. By treatment with nitrous acid, ricinoleic acid is converted into the solid isomer, ricinelaidic acid. Lead ricinoleate is easily soluble in ether.

### Separation of Fatty Acids.

For the analytical separation of saturated from unsaturated fatty acids, Muter's method, based on the solubility of the lead soaps of the latter in ether, is generally used. In order to determine the constituents of a mixture of homologous acids it is usually necessary to

* *Bull. Soc. Chim.*, 1895, [3] 13, 238.

† *Journ. Amer. Chem. Soc.*, 1899, 21, 817.

‡ Darmstaedter and Lifschütz: *Ber. deut. chem. Ges.*, 1896, 29, 1476.

§ *Bull. Soc. Chim.*, 1895, [3], 13, 240.

‖ Goldsobel: *Ber. deut. chem. Ges.*, 1894, 27, 3121. Kasansky: *Journ. prakt. Chem.*, 1900, 62, 363.

fraction the mixture repeatedly either by distillation, precipitation, or crystallization, until each fraction contains only two acids. The proportions of the two constituents can then be found by determining the mean molecular weight of the mixture. Such methods are outlined by Allen (Commercial Organic Analysis, 3d Ed., I., 485–492; II., 240–246) and by Browne, in a paper on the chemistry of butter fat, *Journ. Amer. Chem. Soc.*, 1899, 21, 807.

## CHAPTER IX.

### Oils, Fats, and Waxes — General Methods.

All glycerides of fatty acids are properly known as fats. As a matter of convenience those fats which are liquid at ordinary temperatures are commonly called fatty oils. Fats and fatty oils, therefore, constitute a definite group of compounds, the glyceryl esters of the fatty acids.

Waxes are esters of fatty acids with monatomic alcohols of high molecular weight. Most waxes are solid at ordinary temperatures and the term is sometimes applied to other solids of similar physical properties, solid hydrocarbons, for example, being frequently called mineral waxes.

The term oil has no strict chemical significance, being applied not only to liquid fats but also to substances, such as mineral and essential oils, which are similar in some physical properties but entirely different in constitution. The mineral oils are conveniently treated in connection with the fatty oils in works on technical analysis, because on account of their similar physical properties they may for certain uses be mixed with, or substituted for, the fatty oils and are sometimes found as adulterants of the latter. The same is true of some of the essential oils, notably rosin oil and turpentine.

The methods given in this chapter are described and discussed with special reference to the analysis of solid and liquid fats. In most cases, however, they are also applicable to waxes, non-fatty oils, and technical mixtures.

#### PROPERTIES OF FATS AND FATTY OILS.

Refined fats and fatty oils are usually light yellow to colorless. Vegetable oils are sometimes tinged green by the presence of chlorophyll. Crude oils are often reddish or even dark brown. The characteristic colors, odors, and flavors of natural fats are due to small quantities of substances other than glycerides, and therefore become less perceptible the more thoroughly the oil is refined. It is believed that all of the natural glycerides except butyrin, if obtained absolutely pure, would be colorless, tasteless, and odorless.

The natural glycerides are all lighter than water and insoluble in it. They can take up a very small amount of water, which is given off as steam on heating. The quantity of water which can be held by a fatty oil without causing turbidity is, however, negligible, so that for practical purposes the fatty oils may be considered as immiscible with water.

They dissolve readily in ether, carbon bisulphide, chloroform, carbon tetrachloride, and benzol, and mix with each other in all proportions. With the exception of castor oil and a few other oils characterized by a large proportion of hydroxy-acids, they are sparingly soluble in alcohol or acetic acid, but dissolve readily in petroleum ether and mix in all proportions with mineral oils. Castor oil is readily soluble in alcohol or acetic acid and not readily miscible with petroleum ether or mineral oils.

The natural fats do not distil without decomposition. When decomposed by heating they give off acrolein which is readily recognized by its characteristic irritating odor.

A simple triglyceride is one in which the three acid radicals are of the same kind. A glyceryl ester containing the radicals of two or three different fatty acids is known as a mixed glyceride. Both simple and mixed glycerides have been isolated from natural fats and certain physical differences in fats which contain practically the same acids are now attributed to the presence of mixed glycerides. For discussions of simple and mixed triglycerides, see Lewkowitsch's Oils, Fats, and Waxes, 3d Ed., Chapter I., and recent papers by Hansen,* Holde,† and Kreis and Hafner.‡

## ANALYTICAL METHODS.

The object of an ordinary fat or oil analysis is not so much to separate individual constituents as to determine certain chemical and physical properties which are fairly constant for each variety when pure and are therefore frequently called analytical constants. All solid and liquid fats being essentially mixtures of triglycerides, any differences in chemical and physical properties (except such physical variations as are due to mixed glycerides) must be attributed mainly to the presence of different fatty acids, or of the same acids in different proportions.

* Ueber das Vorkommen gemischter Fettesaüre-Glyceride in theirischen Fette. Dissertation, Rostock, 1902; *Arch. Hygiene*, 1902, 42, 1.

† *Ber. deut. chem. Ges.*, 1902, 35, 4306.

‡ *Ber. deut. chem. Ges.*, 1903, 36, 1123, 2766; *Ztschr. Unters. Nahr.-Genussm.*, 1904, 7, 641.

The principal differences to be expected are: (1) In the mean molecular weight of the acids present or the relative proportions of acids of high and those of low molecular weight; (2) in the relative number of "double bonds" depending upon the proportions of acids of the stearic, oleic, linoleic, and linolenic types; (3) in the proportion of hydroxy-acids present.

Some of the analytical "constants" express direct measures of one of these three properties. Others, especially the physical constants, are influenced by variation in any of these three directions and therefore express no one chemical property but a resultant of all. The "constants" most used may be grouped on this principle as follows:

1. (*a*) Measuring the mean molecular weight.—Saponification or Koettstorfer number. (*b*) Measuring the proportion of acids of high or of low molecular weight.—Hehner number, Reichert-Meissl number.

2. (*a*) Measuring the proportion of unsaturated acids (number of "double bonds").—Hübl number and other halogen absorption numbers. (*b*) Depending mainly upon the proportion of unsaturated acids.—Thermal reactions with bromine or sulphuric acid, Maumené number.

3. Measuring the hydroxyl radical and therefore depending mainly upon the presence of hydroxy-acids.—Acetyl number.

4. Influenced by all of the above properties.—Specific gravity, index of refraction, melting point, "titer test," viscosity, solubilities.

## The Saponification or Koettstorfer Number.*

The saponification or Koettstorfer number is the number of milligrams of potassium hydroxide consumed in the complete saponification of one gram of the fat or wax; or, in other words, it is ten times the percentage of potassium hydroxide required to neutralize the total fatty acids in the sample, whether free or in the form of esters.

*Reagents.*—1. Standard solution of hydrochloric acid preferably half-normal.

2. Alcoholic potash solution containing 40 grams of potassium hydroxide per liter of purified 95 per cent. alcohol. In the prep-

*Koettstorfer: *Ztschr. anal. Chem.*, 1879, 18, 199, 431. Reprinted in Ephraim's Originalarbeiten über Analyse der Nahrungsmittel, Leipzig, 1895.

aration of this solution the best available potassium hydroxide (purified by alcohol) should be dissolved in alcohol which has been purified by redistillation over caustic alkali. The solution must be clear when used.

3. As indicator a 1 per cent. solution of phenolphthalein in purified 95 per cent. alcohol.

*Determination.* — Weigh 4 to 5 grams of the fat or oil in a 250 c.c. Erlenmeyer flask, add 50 c.c. of the alcoholic potash solution, connect with a reflux condenser and boil for thirty minutes, or until the oil is completely saponified, so that the liquid in the flask appears homogeneous and clear. At the same time measure 50 c.c. of the alcoholic potash solution into an empty flask of the same size and shape, connect with a similar reflux condenser and boil for the same length of time as in the case of the solution containing the sample. Cool the flasks and titrate each with the standard hydrochloric acid, using 1 c.c. of the phenolphthalein solution as indicator. The difference between the titrations gives a measure of the potassium hydroxide consumed in saponifying the sample.

*Notes.* — When ordinary alcohol is used for the potassium hydroxide solution, the latter rapidly turns brownish so that the final titration is difficult. Alcohol which has been treated with potassium hydroxide, allowed to stand for one to two weeks and then redistilled, gives a much more permanent solution. According to Gill scarcely any darkening of the solution occurs if it is kept under an atmosphere of hydrogen. Great care must be exercised to measure exactly the same quantity of the alkaline solution into each flask, and to treat the blank solution in exactly the same way as that containing the sample, so that any loss of alkalinity due to absorption of carbon dioxide from the air, or to the possible action of the alkali on the solvent, may be the same in each case. If sulphuric acid were used in place of hydrochloric for the final titration a precipitate of potassium sulphate would be formed in the alcoholic solution, thus impairing the delicacy of the end reaction.

In order to avoid the changes which may occur in the alkali solution on boiling, Henriques * recommends that the saponification be conducted in the cold. From 3 to 4 grams of oil are mixed with 25 c.c. of petroleum ether and 25 c.c. of normal alcoholic potash and allowed to stand over night at room temperature, when the saponification is said to be complete.

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* *Ztschr. angew. Chem.*, 1895, 721; 1896, 221, 423.

*The Saponification Equivalent.*

The results obtained as described are sometimes expressed in terms of the *saponification equivalent.* This is the weight of fat which reacts with the molecular weight of sodium or potassium hydroxide; or, in other words, the number of grams of fat which would be saponified by one liter of normal alkali. Comparing this with the definition of the *saponification* (or Koettstorfer) *number* it will be seen that these two values express the same property in reciprocal terms and that the product of the two values is always equal to the number of milligrams of potassium hydroxide in a liter of normal solution, viz. 56,158.

Hence

$$\text{Saponification equivalent} = \frac{56{,}158}{\text{saponification number}}$$

$$\text{Saponification number} = \frac{56{,}158}{\text{saponification equivalent}}$$

For a sample consisting entirely of triglycerides the saponification equivalent would be exactly one-third of the mean molecular weight of these glycerides.

*Acid and Ester Numbers.*

The *acid number* of a fat or wax is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of substance.* The *ester number* is the difference between the saponification number and the acid number and therefore shows the amount of alkali consumed in the saponification of esters.

To determine the acid number, shake 4 to 5 grams of the sample with 50 c.c. of carefully neutralized alcohol and titrate with tenth-normal or half-normal alkali, using phenolphthalein as indicator. As the oil itself mixes but slightly with alcohol, it is necessary toward the end of the titration to shake thoroughly after each addition of alkali to secure complete extraction of the fatty acid from the oily layer.

* The acidity of a sample of oil or fat is not always expressed as the "acid number." Frequently it is recorded in terms of the equivalent percentage of free oleic acid, "acidity as oleic," and sometimes as "degrees of acidity." The latter term indicates the number of c.c. of normal caustic alkali required to neutralize the free acids in 100 grams of the fat.

## THE HEHNER NUMBER.

The Hehner number is the percentage of insoluble fatty acids obtainable from a fat.

As the determination is ordinarily made, the unsaponifiable matter present in the fat is weighed with the insoluble acids. The great majority of fats and fatty oils have Hehner numbers between 94.5 and 96. Butter fat has a lower Hehner number and the determination of this "constant" is of value chiefly in testing the purity of butter. The detailed description of the process will therefore be given in the chapter on butter analysis.

## THE REICHERT-MEISSL NUMBER.

The Reichert-Meissl number is the number of cubic centimeters of tenth-normal caustic alkali required to neutralize the soluble volatile acids obtained from 5 grams of a fat by the Reichert distillation process.

This number serves as a comparative measure of the acids of low molecular weight. Its principal use is in the examination of butter fat and the detailed description will be given in that connection. The Reichert number is about one-half the Reichert-Meissl number, Reichert having originally recommended the use of 2.5 grams of fat. Among the common oils and fats the Reichert-Meissl numbers are usually less than 1.0. Butter fat has a high Reichert-Meissl number and as this determination is used principally in the examination of butter, it will be described in the chapter on butter analysis.

## THE IODINE OR HÜBL NUMBER.

The iodine or Hübl number is the percentage of iodine (or of iodine chloride or bromide expressed in terms of iodine) absorbed by the sample.

This number gives a quantitative measure of the unsaturated fatty acids (or of the "number of double bonds") in a fat or wax.

Mills, Snodgrass and Akitt were probably the first to make systematic use of the halogen absorbing power in fat analysis. In their experiments* the oil or fat to be tested was dissolved in carbon tetrachloride and titrated with a standard solution of bromine in carbon tetrachloride as long as the bromine was absorbed. At

* *Journ. Soc. Chem. Ind.*, 1883, 2, 435; 1884, 3, 366.

about the same time, Hübl published* a method based upon the use of iodine in an alcoholic solution of mercuric chloride which was so carefully worked out in all of its details that the original form of the process is still used in many laboratories in preference to any of the modifications which have been proposed. Recently, however, the Wijs† and the Hanus‡ modifications have been largely used, and it is probable that they will gradually replace the Hübl process. These three methods will be given here. The bromine absorption method as developed by McIlhiney§ can be used in place of the iodine methods, but is more especially adapted to the examination of linseed oil for rosin oil or rosin, and will be referred to in that connection in the next chapter.

### *Method of Hübl.*

*Reagents.*— 1. Iodine solution. Dissolve 26 grams of pure iodine in 500 c.c. of 95 per cent. alcohol. Dissolve 30 grams of mercuric chloride in 500 c.c. of alcohol of the same strength. Mix the two solutions at least 12 hours before using. As this solution is expensive and does not keep well, no more than a week's supply should be made at one time.

2. Standard solution of sodium thiosulphate. Dissolve 24 grams of the crystallized salt in a liter of water, allow to stand at least 24 hours and then determine the strength of the solution in terms of iodine. While any of the well-known methods may be used, it is convenient to standardize the thiosulphate solution as follows:

Weight 3.8694 grams of pure dry potassium dichromate, dissolve in water and dilute to 1000 c.c. In a well-filled tightly-stoppered bottle, this standard solution of dichromate can be kept indefinitely without deterioration. Each c.c. of this solution is equivalent to 0.01 gram iodine. Mix 25 c.c. of a 15 per cent. potassium iodide solution with 5 c.c. of hydrochloric acid, add 50 c.c. of the dichromate solution, and titrate the liberated iodine by means of the thiosulphate solution, observing procedure and precautions described below. Calculate the amount of iodine consumed by each c.c. of the thiosulphate solution. As a precaution the strength of either the dichromate or the thiosulphate solution should also be determined by an independent method.

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* *Dingl. polyt. Journ.*, 1884, 253, 281. Reprinted by Ephraim, *loc. cit.*

† *Ber. deut. chem. Ges.*, 1898, 31, 750.

‡ *Zts. hr. Unters. Nahr.-Genussm.*, 1901, 4, 913.

§ *Journ. Amer. Chem. Soc.*, 1884, 16, 245; 1899, 21, 1084; 1902, 24, 1109.

3. An approximately 15 per cent. solution of pure potassium iodide in cold, recently boiled, distilled water.

4. Freshly prepared starch solution, 1 part starch to 200 parts of water, for use as indicator.

5. Pure chloroform.

6. Cold, recently boiled, distilled water.

*Determination.* — Thoroughly clean and dry two or more thin Erlenmeyer flasks, of the form made for this purpose, having accurately ground glass stoppers and flaring mouths which form a gutter between the stopper and the lip. Into one of these flasks weigh such an amount of the sample as will absorb between 0.3 and 0.4 gram of iodine * and add 10 c.c. of chloroform. When the sample has completely dissolved, add 25 c.c. or 30 c.c. of the mixed iodine solution, stopper carefully, fill the gutter around the stopper with potassium iodide solution to guard against loss of iodine, shake gently, and allow the flask to stand in a cool dark closet for 3 to 20 hours according to the nature of the sample. In another clean dry flask of the same size and form make a blank determination using the same amounts of chloroform, iodine solution, and potassium iodide. Allow the two flasks to stand side by side for the same length of time. When the absorption is complete, lift the stopper in such a way that its lower surface will be washed by the iodide solution from the gutter; add 100 c.c. of cold, recently boiled, distilled water and 20 c.c. more of the potassium iodide solution, washing down the sides of the flask with the latter. In case a red precipitate of mercuric iodide appears, add more potassium iodide until the precipitate is dissolved. Titrate the excess of iodine at once by means of the standard thiosulphate solution. The latter may be run in rapidly until the iodine is nearly consumed and the solution is only light yellow; then add 2 c.c. of the starch solution and finish the titration carefully but without delay. As the end point is approached, stopper the flask quickly after each addition of thiosulphate and shake vigorously to ensure thorough and rapid mixing of the contents. The end point should be sharper than in standardizing since in this case there is no green color due to chromium and the solution passes at once from blue to nearly colorless. The difference between the volume of thiosulphate solution required for the blank test and that required for the solution containing the sample gives a meas-

* See iodine numbers in table at the end of this chapter.

ure of the iodine absorbed by the latter. Calculate the iodine absorbed in terms of percentage of the original weight of sample.

*Notes.* — Most of the difficulties which are met in using this method are due to impure reagents or failure to observe carefully the conditions worked out by Hübl. Probably the most important sources of error are: (1) The use of impure chloroform or water containing dissolved air causing a liberation of iodine from the potassium iodide; (2) loss of iodine from the use of vessel with imperfectly fitting stopper or from titrating at too high temperature or with too much exposure to air; (3) deterioration of the iodine solutions due chiefly to impurities in the alcohol or too high temperature; (4) variations in the excess of iodine over that absorbed; (5) variations in the length of time allowed for the reaction.

The small amount of oil needed for this determination can conveniently be drawn from near the center of the sample bottle by means of clean glass tubing of about 2 mm. internal diameter. Use a piece of thin tubing like a pipette, wiping it free from oil on the outside and allowing it to deliver drop by drop into the weighed flask which should then be stoppered and reweighed at once. If more than one sample of oil is to be tested, delays can be avoided by having a number of pieces of the tubing cleaned and dried in advance so that each can be rejected after using it for one sample.

The best results are obtained by using such proportions of oil and of iodine solution as to have present from two to three times the amount of iodine which will be absorbed. With samples which absorb only small amounts of iodine the reaction is probably complete in two or three hours, but with linseed and similar oils it is necessary to allow the iodine solution to act longer. It has been claimed that several hours standing introduces an error through the deterioration of the iodine solution in the blanks, but this need not be feared if the reagents are pure and the flasks tightly stoppered and kept in a cool dark place. Unless it is certain that a shorter time will be sufficient it is well to allow the determinations to stand over night. The form of vessel to be used for the test is, of course, immaterial so long as loss of iodine is avoided. Some prefer to weigh the sample on a small watch glass and place the latter with the sample in a wide-mouth glass stoppered bottle. A blank test must be made with each determination, or if several samples are treated at once there should be at least two or three blanks.

For discussion of the theory of the action of Hübl's solution see Lewkowitsch's Oils, Fats, and Waxes, or Gill's Oil Analysis.

*Method of Wijs.*

Wijs found that a solution of iodine monochloride in glacial acetic acid acts in the same manner as Hübl's solution, but more quickly. Moreover, the reagent is much more stable than that of Hübl, so that the same solution can be used for several months and blank tests are not so frequently required.

*Reagents.*— 1. Glacial acetic acid. This must contain not over 0.5 per cent. of water and no impurity capable of reducing potassium dichromate. Test by adding a small amount of a solution of dichromate in sulphuric acid and warming on a water-bath; no green tinge should appear.

2. Iodine chloride solution. Dissolve 12.5 to 13 grams of iodine in a liter of the glacial acetic acid, warming gently to aid solution, if necessary. Cool, withdraw 25 c.c., add potassium iodide solution, and determine the iodine content by titration with standard thiosulphate; into the remainder of the solution pass a current of chlorine until the deep iodine color changes to an orange yellow, showing that the conversion of iodine to iodine monochloride is complete. A portion of the solution titrated with thiosulphate should now show twice the original halogen content. In case an excess of chlorine is found, a weighed amount of iodine equivalent to this excess should be dissolved in the solution.

3. Standard solution of sodium thiosulphate, as in the method of Hübl.

4. Pure chloroform, potassium iodide, starch solution, and distilled water as described under Hübl's method.

*Determination.*—The determination is carried out in exactly the manner described for the Hübl method except that 10 c.c. of the potassium iodide solution are sufficient and a much shorter time is required for the reaction. With non-drying oils the addition of iodine chloride is complete in 15 minutes. Ordinarily the solution should be titrated after standing one hour.

*Notes.*—As the solution of iodine chloride in acetic acid is more viscous and has a higher coefficient of expansion than ordinary aqueous solutions, the portions added must be measured as carefully as possible, allowing a uniform time for the burette to drain, and observing that the temperature does not vary more than three or

four degrees. With these precautions it is not always necessary to make a blank test with each determination after a sufficient number of such tests have been made to show that the solution is not changing appreciably in strength.

*Method of Hanus.*

Hanus suggested that Wijs' method be modified by the use of iodine bromide in place of iodine chloride. As an excess of iodine does no harm, the preparation of the reagent is simpler than in the preceding method.

The reagents required are the same as for the Wijs method except that the iodine bromide solution is prepared by dissolving 13.2 grams of iodine and 3 c.c. of bromine in a liter of glacial acetic acid. The iodine may be dissolved first in the greater part of the acid, heating to aid solution; the bromine is added after cooling the solution and the volume then brought to a liter.

The manipulation is the same as in the Wijs method, but as the reaction is not quite so rapid an hour should be allowed in all cases. It is well to make the determination in duplicate, allowing more time in one case than in the other. After adding the potassium iodide solution shake thoroughly before adding water.

*Comparison of the Hübl, Wijs, and Hanus Methods.*

Wijs based his modification on the belief that iodine chloride is the active substance in Hübl's solution and that the more stable solution of iodine chloride in acetic acid should give the same theoretical results with greater certainty. That higher results were often obtained by his method Wijs attributed to the deterioration or incomplete action of the Hübl solution as ordinarily prepared. Lewkowitsch * confirms this, stating that when the Hübl method is carried out under the best conditions the results of the two methods agree.

Tolman and Munson, † however, in an extended comparison of the three methods almost invariably obtained higher results by the Wijs than by the Hübl method. Hanus' method gave intermediate results, usually agreeing quite closely with the Hübl numbers. Similar results on a smaller number of samples had previously been published by Hunt ‡ and have since been obtained by W.

* *Analyst*, 1899, 24, 259.

† *Journ. Amer. Chem. Soc.*, 1903, 25, 244.

‡ *Journ. Soc. Chem. Ind.*, 1902, 21, 454.

G. Tice.* For oils having iodine numbers below 100, the three methods can be used interchangeably as the differences in results will rarely exceed 1.5 units which is only about one-tenth of the variation which occurs among pure oils of the same species. In applying the Wijs solution to oils having much higher iodine numbers it is to be remembered that results as much as 10 units in excess of these given by Hübl's solution will sometimes be obtained.

The method of Hübl is "official" in the United States but that of Hanus has been adopted by the Association of Official Agricultural Chemists as a provisional optional method for the examination of edible oils and fats. The method of Wijs is more commonly used in England and is recommended in preference to the Hanus method by Lewkowitsch † and Archbutt, ‡ the latter pointing out especially that the Wijs solution gives approximately the theoretical iodine number for turpentine (which is often mixed with fatty oils) while that of Hanus gives much lower results.

In comparing analytical results with the numbers found in "tables of constants" it should be remembered that the latter are based in part upon results obtained (not always under the best conditions) by Hübl's method, and in part upon later results determined according to Wijs. Such tables tend to show a greater range than would be found by the use of either method alone, this being especially true of the oils having high iodine numbers.

### Maumené Number — Specific Temperature Reaction.

Long before the introduction of any of the halogen absorption methods Maumené § tested olive oil for adulterants such as poppy-seed oil by mixing fixed volumes of oil with strong sulphuric acid and observing the rise in temperature which is much greater with seed oils than with olive. The rise of temperature, expressed in degrees C., which occurs on mixing 10 c.c. of strong sulphuric acid with 50 grams of oil is commonly known as the Maumené number. Thomson and Ballantyne ‖ pointed out the discrepancies which may be introduced through variations in the strength of the acid used, and showed that they can be largely avoided by comparing the rise observed in the case of an oil with that

* In the Havemeyer Laboratory, Columbia University.

† Oils, Fats and Waxes (3d Ed.), I., 252.

‡ *Journ. Soc. Chem. Ind.*, 1904, 23, 306.

§ *Compt. rend.*, 1852, 35, 572.

‖ *Journ. Soc. Chem. Ind.*, 1891, 10, 234.

shown by water under the same conditions, the latter being taken as 100. The result thus obtained is known as the specific temperature reaction or specific Maumené number. Thus if 50 grams of water mixed with 10 c.c. of sulphuric acid showed a rise of 40° and 50 grams of an oil showed under the same conditions a rise of 36° the specific Maumené number of the oil would be 90.

With fresh fatty oils the rise of temperature depends mainly upon the presence of glycerides of the unsaturated acids and the Maumené and Hübl numbers are therefore roughly proportional. This relation, however, is not sufficient to permit of the determination of the Maumené in place of the iodine number except for rough work or as a preliminary test. The greatest value of this temperature reaction lies in the fact that as oils become altered by absorption of oxygen from the air, the Maumené numbers increase while the iodine numbers decrease. The significance of this relation is more fully explained in the next chapter.

*Apparatus and Reagents.* — A deep beaker of 150 to 200 c.c. capacity should be used for this test and should be so jacketed as to prevent a rapid loss of heat. This is done by placing it in a larger beaker or a metal cup and filling the space with asbestos or other dry porous material. If a new nest of beakers is at hand the test can be made in one of the smaller beakers using the remainder of the nest with the packing intact as a jacket. The insulation should be sufficient to prevent the outer vessel from becoming perceptibly warm when a test is made. The only reagent required is strong sulphuric acid which can be added either from a burette or (more satisfactorily) from a 10 c.c. pipette. For observing the rise in temperature use a thermometer graduated in degrees on which the readings can be taken with accuracy to 0.2 degree.

*Determination.* — The oils to be tested, the acid and apparatus to be used, and the distilled water which is to serve for comparison must all have the same initial temperature, which should be between 20° and 24°. This will usually be the case if they have stood side by side at room temperature for several hours. Measure 50 c.c. of water into the beaker, introduce the thermometer and note the temperature; add 10 c.c. of the acid, stirring thoroughly with the thermometer so that the solution is kept well mixed and the temperature rises steadily. At intervals of a few seconds bring the bulb of the themometer to the center of the solution and

observe the temperature. Record the highest reading found. When the mixture in the beaker has reached its maximum temperature it should be rejected at once to avoid unnecessary warming of the apparatus, since the initial temperature must be restored before beginning another test. After the rise with water has been found by duplicate determinations which do not differ by more than 0.4°, dry the beaker thoroughly, weigh into it 50 grams of oil (within 0.05 gram), and treat with 10 c.c. of acid in exactly the same manner. The mixture often becomes very viscous, requiring vigorous stirring to ensure a uniform temperature. In reporting results give the actual rise observed with oil and with water as well as the specific temperature reaction.

*Notes.* — By the use of a pipette having a fine outlet requiring 30 to 60 seconds for the 10 c.c. of acid to flow into the beaker, more thorough mixing becomes possible and better results are secured. In order to avoid trouble in cleaning the beaker it should be emptied and wiped with dry cotton waste or porous paper while still warm. The acid used must be protected from unnecessary exposure to air as it readily absorbs moisture to an extent sufficient to cause an appreciably lower temperature reaction. In testing edible and lubricating oils the strongest available sulphuric acid should be used. In the case of drying oils and fish or other marine animal oils, the addition of such strong acid to the undiluted oil causes violent frothing and decomposition. This may be avoided * by the use of sulphuric acid of such strength as to give a rise of 35° when added to water under the conditions to be used in testing the oil. An important modification of this test has been proposed by Mitchell.†

### THE ACETYL NUMBER.

The acetyl number is the number of milligrams of potassium hydroxide required to neutralize the acetic acid obtained by saponification of one gram of the acetylated fat or wax.

It indicates the proportion of alcoholic hydroxyl groups in the original substance. If the latter consists essentially of triglycerides, the acetyl number serves as a measure of the hydroxy-acids present. Free alcohols, if present, increase the acetyl numbers. Lew-

* Sherman, Danziger and Kohnstamm : *Journ. Amer. Chem. Soc.*, 1902, **24**, 266.

† *Analyst*, 1901, **26**, 169. See also Sherman and Falk : *Journ. Amer. Chem. Soc.*, 1903, **25**, 713.

kowitsch finds that free fatty acids of the stearic series may also react in such a way as to increase the apparent acetyl numbers, and therefore prefers to acetylate the original fat or wax rather than to work with the mixed fatty acids as was previously recommended by Benedikt and Ulzer.*

The method of Lewkowitsch is essentially as follows:†

Boil 10 grams of the substance with twice its weight of acetic anhydride in a round bottomed flask under a reflux condenser for two hours; pour the resulting mixture into a large beaker containing 500 c.c. of hot water and boil for half an hour, passing a slow current of carbon dioxide through the solution to prevent bumping. Allow the mixture to separate into two layers, siphon off the water, and boil the oily layer with three successive portions of fresh water, the last of which should not react acid to litmus paper.

All free acetic acid having been removed, the acetylated fat is carefully separated from water and further dried by filtering through anhydrous paper in a drying oven.

Weigh 2 to 5 grams of the acetylated fat and saponify with a measured volume of standard alcoholic potash as in the determination of the saponification number; evaporate nearly to dryness to expel the alcohol, dissolve the soap in water and add an amount of standard sulphuric acid exactly equivalent to the alkali used for saponification. Warm gently until the fatty acids separate as a layer at the top. Filter through wet paper, wash the fatty acids with boiling water until the filtrate is no longer acid, and titrate the filtrate and washings with tenth-normal alkali, using phenolphthalein as indicator. Deduct the amount of alkali required to neutralize any soluble fatty acids in the original substance and calculate the acetic acid found, in terms of acetyl number as defined above.

Instead of filtering and washing with water, the acetic acid can be separated from the fatty acids by distilling with steam, but this requires considerably more time than the filtration process.

In the examination of waxes the acetyl numbers show the proportional amounts of free alcohols. Fats and fatty oils contain free alcohols (cholesterol, phytosterol, etc.), but in very small amounts only, so that the acetyl numbers are low except in oils which contain hydroxy-acids. (Chapter VIII.) The acetyl number of castor oil is about 150. Other oils rarely show acetyl

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* *Monatsh. Chem.* 1887, 8, 40.

† *Journ. Soc. Chem. Ind.*, 1897, 16, 503; Oils, Fats, and Waxes (3d ed.), 268.

numbers higher than 10, unless they have been exposed to atmospheric oxidation, in which case hydroxy-acids may have been formed from the unsaturated acids originally present.

### Specific Gravity.

The specific gravities of the glycerides depend upon the fatty acids which they contain. Comparing acids of the same homologous series the specific gravity was found to decrease with increasing molecular weight; while between corresponding acids of different series it increases with the number of double bonds and of hydroxyl groups.

Fresh specimens of the same kind vary but little in specific gravity, and the determination of this property is often useful in differentiating oils, fats, and waxes and in testing them for adulterations. The determination can be made either by means of a very delicate special hydrometer, by the Westphal balance, or by one of the methods described in Chapter III. On account of the high coefficient of expansion of oils it is important that the temperature at which the specific gravity is taken be accurately known. This is conveniently accomplished by using the thermometer sinker with the Westphal balance.

Directions for the use of the Westphal balance are usually supplied with the instrument. It should always be placed upon a *firm level table and very carefully adjusted before using. In setting* up the instrument be sure that the leveling screw is directly beneath the arm which supports the beam. Place the latter in position and hang the sinker. Compare the height of the beam with that of the cylinder which is to contain the oil and lengthen or shorten the standard if necessary, then carefully adjust the balance by means of the leveling screw until the point projecting from the end of the beam stands exactly opposite the fixed point at the left. Nearly fill the cylinder with the oil at about 14°. Lift the sinker, place the cylinder under the end of the beam and replace the sinker so that it hangs freely in the oil. The specific gravity is now found by placing weights on the beam until the balance is restored. Make the final adjustment of the smallest weight when the thermometer in the sinker shows the desired temperature — for oils, 15.5°. The specific gravity is read directly from the position of the weights on the beam. If the largest weight is at 9, the second at 3, the third at 1, and the fourth at 5, the spe-

cific gravity of the oil is 0.9315. The readings should be made to four decimal places (*i. e.*, all four sizes of weights should be used) and the error of determination should not exceed 0.0005. By using the same sinker on the analytical balance or by weighing the oil in a pyknometer, more accurate results can be obtained. The Westphal balance is, however, sufficiently delicate for routine work if carefully used. Care must be taken that the sinker is always suspended at the same depth in the liquid under examination. The graduation of the beam should be tested to see that the spaces are all of equal length, and the results should occasionally be verified by comparative determinations with the pyknometer. In working with very viscous oils it is advantageous to use a sinker of high specific gravity.*

For the determination of specific gravity at temperatures considerably above that of the laboratory it is convenient to use a Sprengel or Ostwald pyknometer. This is filled with the sample, suspended in water at the required temperature and adjusted, then removed, dried on the outside, cooled to room temperature and weighed. For a detailed description of the standardization of pyknometers at high temperatures consult any standard work on physico-chemical measurements or Bulletin 46 or 65, Bureau of Chemistry, U. S. Department of Agriculture.

## INDEX OF REFRACTION.

The index of refraction, like the specific gravity, increases with the proportion of unsaturated, or of hydroxy-acids. Unlike the specific gravity, the index of refraction increases with the molecular weight in the homologous series of saturated acids so that the presence of the fatty acids of low molecular weight in butter causes this fat to have a lower index of refraction than other animal fats, while its specific gravity is higher. In almost all of the fatty oils the index of refraction varies with the specific gravity so that in routine work it is not necessary to make both determinations but either may be used to confirm the inferences drawn from the other determinations.†

The index of refraction has usually been determined by means of the Abbe or the Pulfrich refractometer. The "oleo-refractome-

* McGill: *Analyst*, 1896, **21**, 156.

† For a very full discussion of the relation of index of refraction to specific gravity in oils and fats see Procter: *Journ. Soc. Chem. Ind.*, 1898, **17**, 1021.

ter " of Amagat and Jean is a differential instrument in which the results are found in terms of an arbitrary scale, the value of which, according to Allen, is not constant. The readings of this instrument cannot, therefore, be compared with determinations of the true index of refraction. The " butyro-refractometer " of Zeiss also has an an arbitrary scale but in this case the values of the scale readings are known and can be compared with direct determinations of the index of refraction as follows:

| Butyro-refractometer Reading. | Index of Refraction. | Butyro-refractometer Reading. | Index of Refraction. | Butyro-refractometer Reading. | Index of Refraction. |
|---|---|---|---|---|---|
| 0 | 1.4220 | 40 | 1.4524 | 80 | 1.4783 |
| 10 | 1.4300 | 50 | 1.4593 | 90 | 1.4840 |
| 20 | 1.4377 | 60 | 1.4659 | 100 | 1.4895 |
| 30 | 1.4452 | 70 | 1.4723 | | |

Since the butyro-refractometer is constructed to cover only such a range in the index of refraction as is likely to be met in work with fats and oils, it is possible to make more delicate readings with this instrument than with the Abbe refractometer. It is preferable, however, to express all results in terms of the index of refraction, especially as some substances frequently mixed with oils and fats are too highly refractive to be examined in the butyro-refractometer.

The index of refraction decreases with rising temperature. The rate of change is nearly constant for the common oils and fats, a rise of 1° causing a diminution of 0.000365 in the index of refraction.*

Directions for the determination of the index of refraction will be found in Kohlrausch's Physical Measurements, Leach's Food Inspection and Analysis, Lewkowitsch's Oils, Fats, and Waxes, or Vaubel's Quantitative Bestimmung organische Verbindungen.

### Melting and Solidifying Points — Titer Test.

The melting point of a fat, or of the mixed fatty acids obtained from it, increases with the mean molecular weight among acids of the saturated series, while in mixtures of acids of the saturated and unsaturated series (or their glycerides) the melting point becomes lower as the proportion of unsaturated compounds increases.

* Proctor: *Journ. Soc. Chem. Ind.*, 1898, 17, 1023. Tolman and Munson: *Journ. Amer. Chem. Soc.*, 1902, 24, 754.

The melting point of a solid fat is best determined by heating a thin disc of the substance suspended in a mixture of water and alcohol as described in Chapter XI. This method being applicable only to substances practically insoluble in alcohol, the melting point of a mixture of fatty acids or of a wax may be taken as follows: *

Draw up the melted fatty acids (or the wax) into a very thin-walled capillary tube 3 to 5 cm. long according to the length of the bulb of the thermometer to be used. Seal one end of the tube and allow the substance to cool on ice for 12 to 15 hours. Attach to the bulb of a delicate thermometer graduated to 0.2°, immerse in water and heat very slowly. The temperature at which the substance becomes transparent is taken as the melting point.

The so-called "titer test" is the determination of the solidifying point of the mixed fatty acids. This test is largely used as a means of determining the suitability of fats for soapmaking and the details of manipulation are now being studied with a view to the adoption of a uniform method.† The Dalican process which has been quite commonly used is fully described by Lewkowitsch.

### Viscosity.

The presence of glycerides of hydroxy-acids, whether natural as in castor oil or artificial as in the "blown" oils of commerce, gives a very high viscosity compared with fatty oils in which hydroxy-acids are absent. Among the latter, viscosities vary as a rule with the melting points of the mixed fatty acids. While the viscosity may aid in the identification of fats and oils in certain cases, its determination is especially important in the examination of lubricating oils and will therefore be discussed in that connection.

### Heat of Combustion.

The heat of combustion of fats and fatty oils is a property as nearly constant as the specific gravity to which it stands in approximately inverse proportion, being lowered by the presence of acids of low molecular weight, highly unsaturated acids, or acids containing hydroxyl groups. The heats of combustion of butter-fat drying oils, castor oil and blown oils are therefore lower than

* U. S. Dept. Agriculture, Bur. Chem., Bul. 65.

† Tolman: Bul. 81, Bur. Chem., U. S. Dept. Agriculture.

those of body fats and such fatty oils as olive, almond, peanut or rapeseed. Waxes and hydrocarbons have higher heats of combustion than the glycerides. The same is true of the alcohols of very high molecular weight such as cholesterol and phytosterol of which the so-called unsaponifiable matter of fats is chiefly composed. With a suitable oxygen calorimeter the heat of combustion can be very accurately determined and the results are often useful in verifying the conclusions drawn from other determinations. For fuller discussions see the *Journal of the American Chemical Society*, 1896, **18**, 178; 1901, **23**, 164; 1902, **24**. 348; 1903, **25**. 659.

## ALCOHOLS OF FATS AND WAXES.
## (UNSAPONIFIABLE MATTER.)

Under the term "unsaponifiable matter" are included all substances found in fats and waxes which are insoluble in water and do not combine with caustic alkali to form soluble soaps. In a commercially pure fat or wax the "unsaponifiable matter" consists chiefly of one or more alcohols of high molecular weight, the more important of which are mentioned below.

The common waxes yield 50 to 55 per cent. of insoluble alcohols while the total amount of unsaponifiable matter in fats is much lower, being usually less than 1 per cent. in animal and less than 2 per cent. in vegetable fats.

### ALCOHOLS OF THE SERIES $C_nH_{2n+2}O$.

*Cetyl alcohol*, $C_{16}H_{34}O$, occurs as palmitate in spermaceti. It is a tasteless and odorless solid, insoluble in water but soluble in alcohol and very easily soluble in ether or benzene. Melting point 50°; specific gravity at 99°, 0.7837.

*Octodecyl alcohol*, $C_{18}H_{38}O$, also occurs as ester in spermaceti. It is similar in properties to cetyl alcohol. Melting point 59°; specific gravity at 99°, 0.7849.

*Myricyl alcohol*, $C_{30}H_{62}O$, occurs as palmitate in beeswax and both free and combined in carnaüba wax. It melts at 85° to 88° and is nearly insoluble in cold, but readily soluble in hot alcohol.

Other alcohols of this series are known but need not be considered here. From the examples given it will be seen that the melting points rise and the alcohols become less soluble with increasing molecular weight. The specific gravity, however, does not decrease as in the case of homologous fatty acids.

CHOLESTEROL AND RELATED ALCOHOLS.

*Cholesterol*, $C_{26}H_{44}O$, is the characteristic constituent of the unsaponifiable matter of animal fats. It is only sparingly soluble in cold dilute alcohol or cold petroleum ether, but dissolves readily in ether, chloroform, carbon bisulphide, fatty oils, turpentine, or hot strong alcohol.

*Phytosterol* and *sitosterol* are alcohols, probably isomeric with cholesterol and resembling it closely in physical properties, which occur in vegetable fats, the latter being found especially in the oils of the cereal grains. The identification of one of these alcohols may therefore aid in determining the origin of an oil or fat or in the detection of vegetable fats present as adulterants in the more expensive fats of animal origin. The identification is best accomplished by converting the alcohol into an ester, the properties of the esters being more distinctive than those of the free alcohols. For further information regarding these alcohols see Lewkowitsch's Oils, Fats, and Waxes (3d Ed.), pages 138–144 and the papers of Bömer,* Burian,† Ritter,‡ and Gill and Tufts. §

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* *Ztschr. Unters. Nahr.-Genussm.*, 1898, **1**, 21, 81, 532; 1899, **2**, 46, 705; 1901, **4**, 865, 1070; 1902, **5**, 1018.

† *Monatsh. Chem.*, 1897, **18**, 551.

‡ *Ztschr. physiol. Chem.*, 1902, **34**, 430, 461; **35**, 550.

§ *Journ. Amer. Chem. Soc.*, 1903, **25**, 251, 254.

## TABLE OF ANALYTICAL PROPERTIES OF TYPICAL OILS, FATS, AND WAXES

The quantitative properties which are most used in identification and testing for adulterants are included in this table. The numbers given are intended to indicate the normal range rather than the extreme limits. Results outside of these limits may be obtained upon pure samples but such discrepancies are *usually due to abnormal quality or to changes resulting from* manipulation or exposure. The figures are taken from the books listed at the end of the chapter, from bulletins and journal articles, and in a few cases from determinations not previously published.

| Substance. | Saponification Number. | Iodine Number. | Specific Gravity at $\frac{15 \text{ to } 15.5^\circ}{15 \text{ to } 15.5^\circ}$. | Specific Gravity at $\frac{99^\circ}{15.5^\circ}$. | Index of Refraction at 60° | Melting Point. | Source |
|---|---|---|---|---|---|---|---|
| Cocoanut oil. | 246–268 | 8– 10 | 0.9245 | 0.863–0.874 | 1.438 –1.443 | 20°– 28° | Seeds of *Cocos nucifera*.* |
| Butter fat. | 220–236 | 26– 38 | 0.936–0.940 | 0.865–0.873 | 1.445 –1.449 | 28 – 35 | Cow's milk. |
| Japan wax (tallow). | 210–238 | 4– 15 | 0.960–1.010 | 0.8755 | 1.450 –1.452 | 45 – 56 | Seed coverings of *Rhus succedanea*.* |
| Palm oil. | 196–206 | 50– 57 | 0.921–0.925 | 0.857–0.860 | 1.450 –1.452 | 27 – 43 | Fruit of *Elaeis guineensis*.* |
| Cacao butter. | 192–202 | 32– 42 | 0.945–0.980 | 0.858 | 1.449 –1.451 | 24 – 34 | Seeds of *Theobroma cacao* (cocoa nibs). |
| Oleo oil. | 193–200 | 40– 55 | 0.925–0.930 | 0.858–0.863 | 1.452 –1.454 | 33 – 39 | Pressed from beef tallow. |
| Beef tallow. | 193–198 | 35– 45 | 0.925–0.929 | 0.860 | 1.449 –1.452 | 42 – 49 | Body fat of cattle. |
| Mutton tallow. | 192–196 | 32– 45 | | 0.858–0.860 | 1.451 | 44 – 50 | Body fat of sheep. |
| Lard. | 193–198 | 50– 70 | 0.931–0.936 | 0.859–0.864 | 1.450 –1.454 | 36 – 46 | Body fat of swine. |
| Cottonseed stearin. | 193–196 | 88–104 | 0.919–0.923 | 0.864–0.869 | | 26 – 40 | Seeds of *Gossypium herbaceum*. |
| Spermaceti. | 122–136 | 0– 5 | 0.905–0.960 | 0.808–0.816 | | 42 – 49 | Head and blubber of sperm whale. |
| Beeswax. | 87–107 | 7– 11 | 0.959–0.975 | 0.818–0.827 | 1.447–1.449† | 61 – 70 | Honey combs. |
| Carnaüba wax. | 78– 85 | 13.5 | 0.990–0.999 | 0.8422 | 1.462–1.465† | 83 – 91 | Leaves of *Corypha cerifera*. |
| Paraffin.‡ | 0 | | 0.868–0.915 | 0.748–0.760 | 1.433–1.436† | 52 – 55 | Petroleum, ozokerite. |
| Rosin (colophony). | 175–195 | 140–160 | 1.04 –1.11 | | 1.548§ | 80 –100 | Wood of several species of pine. |

* Obtained also from other species of the same genus.

† Determined at 65° to 90°. Results are calculated to 60° for convenience of comparison.

‡ Such as is likely to be mixed with beeswax.

§ Temperature of observation not known.

| Oil. | Saponification Number. | Iodine Number. | Specific Gravity at $\frac{15.5^{\circ}}{15.5^{\circ}}$ | Index of Refraction at 15.5° | Specific Temperature Reaction. | Heat of Combustion Calories Per Gram. | Source—(German Name)—"Trade Name" |
|---|---|---|---|---|---|---|---|
| Linseed. | 188–196 | 170–200 | 0.931–0.937 | 1.482 –1.485 | 270–350 | 9,375 | Seeds of *Linum usitatissimum*—flax. |
| Tung. | 190–197 | 150–170 | 0.934–0.942 | 1.5042 | 290–370 | | Seeds of Chinese and Japanese trees—"wood oil." |
| Hemp. | 190–195 | 140–160 | 0.925–0.931 | 1.478 –1.480 | 230–285 | | Seeds of *Cannabis sativa* (Hanfoel). |
| Menhaden. | 187–193 | 140–180 | 0.927–0.933 | 1.480 | 305–360 | 9,366 | Bodies of menhaden often mixed with other fish. |
| Codliver. | 175–190 | 135–180 | 0.922–0.933 | 1.479 –1.485 | 240–280 | 9,436 | Livers of cod and dorsch. |
| Seal. | 184–196 | 125–155 | 0.922–0.930 | 1.479 –1.483 | 210–260 | 9,424 | Blubber of seals (Robbenthran, Seehundsthran). |
| Whale. | 188–194 | 106–135 | 0.918–0.931 | 1.476 | 157 | 9,473 | Blubber of whales (Walfischthran)—"train oil." |
| Poppy. | 189–197 | 133–143 | 0.923–0.927 | 1.476 –1.478 | 200–240 | 9,382 | Seeds of *Papaver somniferum* (Mohnoel). |
| Sunflower. | 188–196 | 104–135 | 0.920–0.927 | 1.474 –1.478 | 167 | | Seeds of *Helianthus annuus.* |
| Maize. | 188–194 | 111–124 | 0.921–0.926 | 1.4755–1.477 | 160–200 | 9,423 | Seed germs of *Zea mays*—"corn oil." |
| Sesame. | 187–195 | 102–115 | 0.920–0.925 | 1.474 –1.4765 | 140–175 | 9,395 | Seeds of *Sesamum orientale.** |
| Cottonseed. | 190–197 | 102–115 | 0.920–0.925 | 1.4735–1.476 | 140–175 | 9,396 | *Gossypium herbaceum* (Baumwollsamenoel). |
| Mustard. | 170–180 | 92–115 | 0.912–0.920 | 1.474 –1.4765 | 130–190 | | Fatty oil of black and white mustard seeds. |
| Rape (colza). | 170–180 | 92–109 | 0.912–0.920 | 1.474 –1.476 | 125–155 | 9,476 | Seeds of *Brassica campestris* (Rüboel). |
| Almond. | 188–195 | 93–102 | 0.915–0.920 | 1.4725–1.475 | 95–120 | 9,454 | Sweet and bitter almonds (Mandeloel). |
| Arachis. | 186–196 | 85–105 | 0.915–0.921 | 1.471 –1.475 | 105–140 | 9,412 | Peanuts, earthnuts—*Arachis hypogaea.* |
| Olive. | 186–196 | 78–90 | 0.915–0.918 | 1.470 –1.472 | 90–110 | 9,454 | Fleshy fruit of *Olea europaea.* |
| Lard oil. | 190–198 | 65–82 | 0.915–0.918 | 1.469 –1.472 | 80–100 | 9,449 | Pressed from lard. |
| Neatsfoot. | 190–198 | 65–75 | 0.914–0.918 | 1.468 –1.473 | 70–100 | | Feet of cattle. |
| Castor. | 173–186 | 82–90 | 0.960–0.970 | 1.479 –1.481 | 84–94 | 8,849 | Seeds of *Ricinus communis*—castor beans. |
| Sperm. | 125–145 | 78–90 | 0.875–0.885 | 1.465 –1.468 | 100 | 9,946 | Head and blubber of sperm whale. |
| Rosin oil. | 10–35 | 40–80 | 0.966–0.990 | 1.535 –1.550 | 72 | 10,145 | Distilled from crude rosin. |
| Mineral oil. | 0 | 0–15 | 0.78 –0.92 | 1.434 –1.507 | 0–20 | 10,700–11,200 | Burning and lubricating oils from petroleum. |

* Obtained also from other species of the same genus.

## CHAPTER X.

### Fatty Oils — Special Methods.

#### SALAD OILS.

Most salad oils are sold as olive oil. The principal substitutes and adulterants are cotton seed, arachis (peanut), sesame, rape, maize, poppyseed, and lard oils. Both quantitative and qualitative methods must be used in any thorough examination of an oil for adulterants. As a rule an oil should be pronounced adulterated only when quantitative determinations yield results which could not be obtained from a pure oil of normal character. Qualitative tests are usually required to show which of several possible adulterants is present. As yet certain color reactions are indispensable for this purpose, but in using these tests and interpreting the results it must be remembered that they depend upon the presence of constituents which may be removed or destroyed by improved methods of refining, and that olive oil which has been altered by long exposure to air in loosely stoppered or partially filled vessels, or oil containing a small amount of some accidental impurity, may give a reaction which cannot be distinguished from that of the adulterant sought. The results of even the best of the color reactions must therefore be interpreted with great caution and must usually be regarded as of much less significance than the quantitative numbers.

On the other hand a good grade of arachis oil, or a carefully prepared mixture of lard oil with one of the seed oils, can be added to olive oil in large proportion without affecting the ordinary constants to such an extent as to pass the limits which can fairly be regarded as normal. It is necessary, therefore, not only to compare each number found with the established limits for pure oil, but also to view the quantitative results in their relations to each other and to the indications of the qualitative tests.

While the system to be followed and the number of tests required will naturally vary in different laboratories the following may be recommended. Determine accurately the specific gravity, the iodine number, and saponification number. If an abundance of the sample is at hand determine the specific temperature reaction and the acidity. The index of refraction may be found, if

convenient, but is usually of less importance than the other determinations. Apply the nitric acid test, Halphen's test, one or more of the color reactions for sesame oil, and examine for arachidic acid by Renard's method. Finally, if the importance of the sample justifies the time required, separate and examine the unsaponifiable matter and the mixed fatty acids and determine the viscosity of the soap solution by Abraham's method described below.

### Analytical Properties of Olive Oil.

The numbers included in the table of "constants" already given are intended to cover the range of normal variations in oils found in the American markets. Müntz, Durand and Milliau * examined samples from Africa, Spain, Portugal, Greece, Turkey, and the Levant without finding any significant variation in the specific gravity, iodine number, or temperature reaction. According to *Milliau, Bertainchand and Malet,* † *however, Tunis oils have high* specific gravities, the average of 49 samples being 0.9183. Tolman and Munson have recently examined a large number of olive oils many of which were of known origin. The following are taken from their results. ‡

| Description. | | Iodine Number. | Specific Gravity $\frac{15.5^\circ}{15.5^\circ}$. | Index of Refraction 15.5°. | Specific Temperature Reaction. | Free Acid as Oleic Per Cent. |
|---|---|---|---|---|---|---|
| California oils of known origin (42 samples). | Max. | 89.8 | 0.9180 | 1.4718 | 109.7 | 8.21* |
| | Min. | 78.5 | 0.9162 | 1.4703 | 94.5 | 0.20 |
| | Avg. | 85.3 | 0.9170 | 1.4713 | 101.8 | 1.20 |
| Italian oils of known origin (17 samples). | Max. | 86.1 | 0.9180 | 1.4713 | 104.7 | 2.79 |
| | Min. | 79.2 | 0.9155 | 1.4705 | 95.6 | 0.57 |
| | Avg. | 81.6 | 0.9163 | 1.4709 | 99.1 | 1.11 |
| Italian oils (commercial) not found adulterated (57 samples).† | Max. | 84.5 | 0.9179 | 1.4712 | 108.4 | 5.30 |
| | Min. | 77.5 | 0.9150 | 1.4701 | 88.4 | 0.72 |
| | Avg. | 80.9 | 0.9161 | 1.4706 | 97.8 | 2.42 |
| French oils (commercial) not found adulterated (60 samples). | Max. | 85.0 | 0.9183‡ | 1.4713 | 114.4 | 3.63 |
| | Min. | 79.0 | 0.9150 | 1.4699 | 90.4 | 0.45 |
| | Avg. | 81.3 | 0.9166 | 1.4708 | 100.1 | 1.59 |

* Two samples with larger amounts of free acid were found but were excluded from average as being unfit for use as salad oils.

† Omitting one sample containing 15.25 per cent. of free acid and having a specific gravity of 0.9134.

‡ Omitting one sample having an abnormally high specific gravity (0.9196) which may have been due to exposure.

* Bulletin du Ministère de l'Agriculture, 1895. Quoted from Bul. 77, Bur. Chem., U. S. Dept. Agriculture.

† Bulletin de l'Agriculture et Commerce de Tunis. Quoted from Bul. 77, *loc. cit.*

‡ Bul. 77, Bur. Chem., U. S. Dept. Agriculture.

The average iodine number of the oils from California is therefore higher than that of the French or Italian oils and, as might be expected, the higher iodine number is accompanied by a higher specific gravity, refractive index, and temperature reaction. Although individual samples will show slight variations, the same general relation is found on comparing oils which differ in iodine numbers though obtained from the same locality. Thus taking at random, from among the California oils of known origin examined by Tolman and Munson, 10 samples with high and 10 with low iodine numbers the following average figures were found:

| | Iodine Number. | Specific Gravity $\frac{15.5^\circ}{15.5^\circ}$ | Index of Refraction 15.5°. | Specific Temperature Reaction. |
|---|---|---|---|---|
| First group. | 88.44 | 0.9172 | 1.4716 | 105.7 |
| Second group. | 83.12 | 0.9166 | 1.4711 | 98.6 |

The normal relations of the constants to each other and the changes which may occur as the result of age or exposure * must always be taken into consideration when interpreting the results of an analysis.

## Detection of Cottonseed Oil.

The presence of cottonseed oil in olive oil raises the specific gravity, iodine number, and temperature reaction and lowers the viscosity of the soap solution. A mixture of cottonseed and lard oils may, however, be added to olive oil in large quantity without greatly affecting any but the last of these "constants." The qualitative tests for cottonseed oil are therefore of considerable importance.

### *Halphen's Reaction.*†

Dissolve 1 part of sulphur in 100 parts of carbon bisulphide and mix the solution with an equal volume of amyl alcohol.

Mix equal volumes, 2 to 3 c.c. each, of the reagent and the oil to be tested and heat the test tube containing the mixture gently at first until violent boiling ceases, then in a bath of boiling saturated solution of common salt. Heat for 2 hours unless a color develops sooner. If cottonseed oil is present the solution turns orange or red.

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* See section on this subject beyond.

† Halphen: *Ann. Chim. Analyt.*, 1898, 3, 9; *Analyst*, 1898, 23, 131; Bul. 65. Bur. Chem., U. S. Dept. Agriculture.

This is probably the most sensitive and characteristic test for cottonseed oil and the least liable to give unsatisfactory results in the hands of an inexperienced person. The presence of 1 or 2 per cent. of unchanged cottonseed oil in olive oil is detected without difficulty. A reaction is often obtained * with lard or lard oil or even with butter fat † from animals which have been fed upon cottonseed meal. Copac (or kapok) oil, which is closely related to cottonseed oil, gives the same reaction. When heated at 250° for 10 to 20 minutes, cottonseed oil loses the property of giving this reaction. No pure olive oil has yet been known to give a similar coloration. Hence a positive result is considered conclusive but a negative result is not. The substance to which the reaction is due cannot be removed by treatment with animal charcoal ‡ and is supposed to be an unsaturated acid which combines with sulphur giving a red compound. §

*Nitric Acid Test.*

Cottonseed oil shaken at room temperature with an equal volume of nitric acid, of 1.37 to 1.38 specific gravity, gives a brown coloration, sometimes only on standing over night. Other seed oils give similar reactions. Normal olive oil under the same treatment shows no change of color.

The test is not so delicate as that of Halphen but is applicable to cottonseed oil which has been heated until it no longer colors the Halphen reagent. It may also be of value in determining whether a weak test with the latter reagent is due to a small amount of unheated cottonseed oil or to a larger amount which has been heated sufficiently to weaken the Halphen reaction (Tolman and Munson). According to Lewkowitsch this reaction cannot be relied upon to detect less than 10 to 20 per cent. of American cottonseed oil in olive oil. Tolman and Munson consider the test much more delicate. A positive result should always be confirmed by finding a high iodine number or by proving the presence of some other oil having a very low iodine number; for pure olive oil if much altered as the result of age and exposure will some

* Soltsein: *Ztschr. öffentl. Chem.*, 1901, 7, 140.

† Wauters: *Bull. Assoc. Belg. Chem.*, 13, 404; *Journ. Soc. Chem. Ind.*, 1900 19, 172.

‡ Utz: *Chem. Rev. Fett. und Harz. Ind.*, 1902, 9, 125; Gill's Oil Analysis, p. 73.

§ Raikow: *Chi... Ztg.*, 1900, 24, 562, 583; 192, 26, 10. See also Halphen: *Bull. Soc. Chem.*, 1905, [3], 33, 108.

times give a reaction which cannot be distinguished from that of cottonseed oil.

## Detection of Arachis (Peanut) Oil.

Arachis oil has usually a higher specific gravity and iodine number and practically always a higher temperature reaction than olive oil. The specific temperature reaction of arachis oil with concentrated sulphuric acid is usually 40 to 60 units higher than the iodine number of the same sample, whereas olive oil usually shows a difference of less than 20 and very rarely of more than 25 units between the iodine and the specific Maumené numbers. The presence of peanut oil in olive oil greatly diminishes the viscosity of the soap solution obtained on saponification. Any of these changes, however, might be due to other adulterants. Arachis oil is shown conclusively by isolating and identifying arachidic acid. This can be done with approximately quantitative results by Tolman's modification of Renard's method.

### *Renard-Tolman Test for Arachidic Acid.* *

Weigh 20 grams of oil in an Erlenmeyer flask. Saponify with alcoholic potash, neutralize exactly with dilute acetic acid, using phenolphthalein as indicator, and wash the solution in a 500 c.c. flask containing a boiling mixture of 100 c.c. of water and 120 c.c. of a 20 per cent. lead acetate solution. Boil one minute and then cool by immersing the flask in water, occasionally giving it a whirling motion to cause the precipitated lead soaps to stick to the sides of the flask. After thorough cooling, pour off the water containing the excess of lead acetate and wash the soap with cold water and then with 90 per cent. alcohol. After pouring off the alcohol as completely as possible, add 200 c.c. of ether, cork the flask and allow to stand until the soap is disintegrated, then connect with a reflux condenser, heat gently to boiling and boil for 5 minutes on a safety water-bath or an electric heater. Cool to 15° and allow to stand over night.

Filter, wash the residue thoroughly with ether, and then transfer it from the filter to the flask by means of a stream of hot water acidified with hydrochloric acid. Add an excess of dilute hydrochloric acid and 200 c.c. of hot water and heat until the fatty acids

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* Renard: *Compt. rend.*, 1871, 73, 1330.

Tolman: U. S. Dept. Agriculture, Bur. Chem., Bul. 81, p. 64. See also Archbutt: *Journ. Soc. Chem. Ind.*, 1898, 17, 1124.

separate as a clear oily layer. Nearly fill the flask with hot water and allow to stand at room temperature until the layer of fatty acids has completely separated and solidified. Remove and drain the cake of fatty acids, wash again with hot water, then dissolve in 100 c.c. of boiling alcohol, 90 per cent. by volume. Cool the solution to 15°, shaking frequently, and allow it to stand as long as any acid continues to crystallize out, or over night, at a temperature not above 20°. Filter, wash the crystals twice with 10 c.c. of 90 per cent. alcohol, noting the total volume of filtrate and washings, and then with alcohol, 70 per cent. by volume (in which the crystals are practically insoluble). Dissolve the crystals by means of hot absolute alcohol in a weighed dish, evaporate, dry, and weigh. To this weight add 0.0045 gram for each 10 c.c. of 90 per cent. alcohol in the filtrate and washings if the temperature of filtration was 20°; or 0.0025 gram for each 10 c.c. if the temperature was 15°.

The melting point of arachidic acid obtained in this way is 71° to 73°. According to Tolman and Munson * the determination of the melting point must not be neglected since cottonseed and lard oils have been found to give crystals resembling arachidic acid in appearance, but having a lower melting point. Tolman finds that from 5 to 10 per cent. of the oil can be detected by this method. On the usual assumption that the oil yields 5 per cent. of the acid,† each centigram found as described above (using 20 grams of oil) indicates 1 per cent. of arachis (peanut) oil in the sample.

## Detection of Sesame Oil.

Sesame oil affects the usually determined constants in the same way as cottonseed oil and to practically the same extent. The color reactions are usually considered quite characteristic.

### *Boudouin's Test.*

Dissolve 0.1 gram of sugar in 10 c.c. of hydrochloric acid of 1.18 to 1.20 specific gravity and add 20 c.c. of the oil. Shake thoroughly in a test tube for one minute and allow to stand. The water solution separates quickly and shows a distinct red or crimson color if the sample contains 1 per cent. or more of sesame

* U. S. Dept. Agriculture, Bur. Chem., Bul. 77, p. 35.

† This is probably more nearly a maximum than an average yield. Tolman and Munson (*loc. cit.*) obtained from 3.41 to 4.24 per cent.

oil. The active reagent is probably furfurol formed by the action of the acid upon the sugar.

*Villivecchia's modification* consists in shaking 10 c.c. of the oil with 10 c.c. of acid (1.20 sp. gr.) to which has been added 0.1 c.c. of a 2 per cent. solution of furfurol in 95 per cent. alcohol.

Olive oils of known purity have usually been found to give only a slight pink color, but sometimes the reddening of the water solution is so pronounced as to cause confusion with that produced by a small amount of sesame oil. Check experiments therefore should always be made. If much sesame oil is present the red color should be perceptible in the oily layer as well as in the water solution.

*Tocher's Test.*

Dissolve 1 gram of pyrogallol in 15 c.c. of concentrated hydrochloric acid. Shake this solution with 15 c.c. of oil in a separatory funnel and allow to stand for 1 or 2 minutes. Draw off the aqueous solution and boil for 5 minutes. The presence of sesame oil is indicated if the solution after boiling appears red by transmitted and blue by reflected light.

This test has not been so generally used nor so thoroughly studied as the preceding. The Association of Official Agricultural Chemists authorize the use of either Boudouin's, Villivecchia's, or Tocher's test for the detection of sesame oil in edible oils and fats.

DETECTION OF RAPESEED OIL.

The acrid taste of this oil would prevent the use of any but highly refined grades in salad oils and since, in America at least, refined rape oil is little if any cheaper than olive, it is not likely to be largely used as an adulterant. The presence in the oil of rape, and other seeds of the *Cruciferæ*, of large proportions of an acid (erucic) having a much higher molecular weight than oleic and linoleic, results in a low saponification figure and a high index of refraction together with low specific gravity. The determination of these constants would probably show rape oil in olive oil if 20 per cent. or more were present.

Rapeseed oil is less soluble than olive oil in acetic acid, but mixtures containing 20 to 40 per cent. of rape oil cannot be distinguished from olive oil by this test (Tolman).

Color reactions for rapeseed oil have been proposed but have not given satisfactory results.

### Detection of Maize, Poppyseed, and Lard Oils.

Each of these oils has a characteristic odor or taste, the odor of lard oil being intensified by heating. These properties, however, cannot be relied upon as the substances to which they are due can be almost entirely eliminated in the refining process. The effect of maize or poppy oil in raising the iodine number would be very noticeable but might be neutralized by the addition of a somewhat greater quantity of lard oil. The difference between the iodine number and the specific temperature reaction would be appreciably greater in such a mixture than in pure olive oil (compare detection of arachis oil). Maize and poppyseed oils react with nitric acid giving brown colors similar to that produced by cottonseed oil. Lard oil often gives the same reaction and might be indicated by the melting point of the fatty acids (those of lard oil having a relatively high melting point, 33° to 38° according to Tolman and Munson) or by the character of the unsaponifiable matter — phytosteryl acetate test.*

All of these oils (as well as most others) yield soap solutions of much lower viscosity than those obtained from pure olive oils.† The determination and significance of this property has recently been studied in some detail ‡ and the method has been found capable of giving valuable results, especially if the conditions worked out by Abraham are carefully observed. For a full discussion of these conditions the original paper must be consulted. The essential features of the process are as follows:

#### *Abraham's Modification of Blasdale's Viscosity Test.*

The saponification number having been determined, weigh 3 grams of oil in an accurately graduated 100 c.c. flask, add 2 c.c. of alcohol and an amount of standard potash solution sufficient to saponify the oil and leave an excess of 0.625 gram of potassium hydroxide. Close the flask with a stopper having a Kroonig valve and saponify on a water-bath. After saponification expel the alcohol by warming and allowing air freed from carbon dioxide to pass through the flask, while a partial vacuum is maintained by means of a suction pump. In this way the alcohol is entirely removed in 5 to 10 minutes. Evaporation should not be carried to com-

*Bömer: *Ztschr. Unters. Nahr.-Genussm.*, 1901, 4, 1091.

† Blasdale: *Journ. Amer. Chem. Soc.*, 1895, 17, 937.

‡ Abraham: *Ibid.*, 1903, 25, 968. Sherman and Abraham: *Ibid.*, 1903, 25, 977.

plete dryness. Without allowing the flask to cool, add 50 c.c. of hot water, rotate gently until a homogeneous solution of the soap is obtained, cool to 20°, fill to the mark with distilled water and mix well by shaking or by repeatedly pouring the solution from one flask to another. Determine the viscosity of the solution in an Ostwald viscosimeter immersed in water kept at 20°. Care must be taken to avoid the introduction of air bubbles into the viscosimeter and to maintain the exact temperature. Repeat the readings until five or more concordant results are obtained. The viscosimeter is standardized by means of distilled water and it is advisable to select for this work an instrument in which the time of flow of water is about 100 seconds. Successive readings of a soap solution should then agree within 2 seconds.

The viscosity is calculated as follows:

$$\eta_1 = \left(\frac{s_1}{t}\right) t_1$$

where

$\eta_1$ = the viscosity number.
$t_1$ = the time of flow (in seconds).
$s_1$ = specific gravity of the solution.
$t$ = time of flow of distilled water.

The viscosity numbers obtained by this method were:

| | |
|---|---|
| Olive oil of known purity (9 samples) | 168.0–185.7 |
| Olive oil of doubtful purity (4 samples) | 145.8–165.8 |
| Lard oil (5 samples) | 122.9–135.0 |
| Arachis (peanut) oil (1 sample) | 126.6 |
| Cottonseed oil (3 samples) | 126.6–127.9 |
| Rapeseed oil " | 124.7–125.7 |
| Sesame oil (1 sample) | 139.2 |
| Maize oil " | 126.6 |
| Poppyseed oil " | 123.9 |

Mixtures of olive and lard oils gave viscosity numbers agreeing closely with those obtained by calculation, but cottonseed or arachis oil when added to olive oil lowered the viscosity to a much greater extent than would have been predicted, indicating that the viscosity number is a more useful means of detecting adulteration than appears from a comparison of the results obtained on testing the olive oil and its adulterants separately.

The usefulness of the method for testing isolated samples is limited by the fact that comparable results can be obtained only

under strictly uniform conditions, the viscosity of the soap solution being greatly influenced by slight variations in strength, alkalinity, or temperature, while the terms in which the results are expressed will naturally vary with the form of viscosimeter used; but in cases of sufficient importance to justify the time required to arrange the apparatus and make comparative determinations, the "viscosity number" will be found an important factor in the examination of olive oil for adulterants.

### DRYING OILS.

While other oils, including especially poppyseed and tung oils, are used as drying oils for special purposes, linseed oil is of much greater importance, being preferred in the great majority of cases in which a drying oil is needed. Only linseed oil and its adulterants will be considered here.

### Analytical Properties of Linseed Oil.

Commercial linseed oil is usually designated by the region of its origin. It varies considerably, the variation being due mainly to the presence of foreign seeds in the linseed at the time of pressing. Hempseed is practically always present, sometimes in very small proportion but often to the extent of 5 per cent., or more, of the weight of seed. The drying properties of linseed oil are better the purer the seeds from which it is pressed. The iodine number and the drying power of the oil decrease as the proportion of hempseed increases. Hence a linseed oil containing much hemp oil would be shown to be of inferior quality by its low iodine number but could not be pronounced adulterated so long as this did not fall below 170. The maximum iodine number of linseed oil is difficult to fix since the results obtained by the method of Wijs often exceed the Hübl numbers, but since no other common oil has a higher iodine number than linseed, the maximum limit is of little practical importance in the detection of adulterations.

The usual range of the more important analytical constants of linseed oil has been given in the table at the end of the preceding chapter. The interpretation of "constants," their relations to each other, and their use in the detection of adulterations having been discussed in some detail in connection with the examination of salad oils, it will be sufficient in this case to mention briefly the principal adulterants with means for the detection of each, and describe the hexabromide test which distinguishes linseed from practically all other oils.

## Adulterants and Methods of Detection.

### *Mineral Oil.*

Mineral oil would greatly lower the iodine number, temperature reaction, and saponification number. Whenever a low saponification number is found the unsaponifiable matter should be separated and examined. Any mineral oil which is not volatile at 100° can be separated quantitatively, dried, and weighed. Volatile mineral oil can be distilled by means of a current of steam, separated from water in the distillate, and measured or weighed. In case turpentine were present, as in some so-called "boiled" oils, it would be distilled with steam and would separate from water in the distillate in the same way. The optical rotatory power of turpentine affords an easy means of distinguishing it from benzine or other volatile mineral oil.

### *Rosin and Rosin Oil.*

Rosin dissolved in linseed oil raises the specific gravity and index of refraction while the saponification and iodine numbers are appreciably decreased only when large amounts of rosin are added. Presence of rosin greatly increases the acid number which in pure linseed oil is usually less than 7. Rosin acids can be separated and determined by Twitchell's method as described under soap analysis beyond.

Rosin oil in linseed oil would raise the specific gravity and greatly lower the saponification and iodine numbers. Rosin oil is a mixture of substances many of which are unsaponifiable, so that its presence in linseed oil would increase the amount of unsaponifiable matter.

Either rosin or rosin oil can be detected by the Liebermann-Storch color reaction or by determining the bromine substitution number.

*Liebermann-Storch Reaction.* — Shake 2 c.c. of the oil with 5 c.c. of acetic anhydride, warming gently. Allow to cool, draw off the anhydride and test by adding one drop of sulphuric acid (1 : 1). A violet color (not permanent) is produced in the presence of rosin or rosin oil. Cholesterol, which might be found in linseed oil if fish oil were present as an adulterant, gives a similar color reaction.

*Bromine Substitution Number* (McIlhiney). — Fatty oils take up bromine by direct addition, little or no substitution taking place. With rosin and rosin oil much the greater part of the bromine is

taken up by substitution, a molecule of hydrobromic acid being formed for each molecule of bromine which disappears. The hydrobromic acid thus affords a means of measuring the amount of substitution. It is determined by adding an excess of potassium iodate and titrating the liberated iodine. The same apparatus can be used as in the determination of the iodine number and the manipulation is similar. From 0.2 to 0.3 gram of the drying oil is dissolved in 10 c.c. of carbon tetrachloride and 20 c.c. of a one-third normal solution of bromine in carbon tetrachloride is added. After two minutes potassium iodide is added and the excess of halogen titrated by means of thiosulphate as in the determination of the iodine number. This shows the total amount of bromine which has disappeared. As soon as this titration is finished, add 5 c.c. of a 2 per cent. solution of potassium iodate and titrate the iodine set free from the iodate by the action of the free halogen acids, according to the reaction : $6HI + KIO_3 = 3I_2 + KI + 3H_2O$.

The bromine thus found is equal in amount to that which has combined with the sample by substitution. For further details of manipulation the reader must be referred to the original papers.* According to McIlhiney the bromine substitution number of raw or boiled linseed oil is always less than 7 while rosin oil gives numbers from 40 to 100 and rosin from 65 to 80.

### *Maize Oil.*

The presence of maize oil in linseed oil lowers the specific gravity, index of refraction, iodine number, and temperature reaction. The amount which can be added without carrying these numbers below the normal limits of variation will depend upon the quality of the linseed oil in the mixture. Since the maize oil used as an adulterant of linseed would probably not be highly refined, the characteristic odor and taste would aid in its detection.

### *Cottonseed Oil.*

The " constants " of linseed oil would be lowered by cottonseed in the same way as by maize oil and to a somewhat greater extent. If the cottonseed oil had not been heated, its presence would be detected by the Halphen test as described under salad oils.

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* McIlhiney : *Journ. Amer. Chem. Soc.*, 1894, 16, 275 ; 1899, 21, 1084 ; 1902, 24, 1109. See also Tolman : *Ibid.*, 1904, 26, 826.

### *Fish Oils.*

Menhaden and other fish oils are often used as adulterants and are difficult to detect with certainty since their "constants" are frequently within the limits found for pure linseed oil. Their presence is often indicated by the odor, but this cannot be relied upon as the difference in odor between refined menhaden and low grade linseed oil is not so pronounced as might be supposed. Lewkowitsch recommends the determination of the melting point of the phytosteryl acetate obtained from the oil, and also that of the hexabromide of the fatty acids (see below). The crystals of phytoseryl acetate from pure linseed oil melt at 128°–129° (Bömer and Winter) while in the presence of cholesterol from fish oil much lower melting points are obtained.

### Hexabromide Test.

Hehner and Mitchell * showed that linseed and fish oils differ from other oils in yielding considerable quantities of insoluble hexabrom-addition products when treated with bromine in ether solution. They applied the test directly to the oil as follows:

Dissolve 1 to 2 grams of oil in 40 c.c. of ether acidulated with glacial acetic acid, cool the solution to 5° and add bromine, drop by drop, until the solution is permanently colored brown. After standing for 3 hours, filter on asbestos and wash successively with 5 c.c. each of cold glacial acetic acid, alcohol, and ether. Dry the precipitate at 100° and weigh.

Linseed oil yields 23 to 38 per cent. of hexabromide, the amount increasing with the iodine number of the oil. Some of the fish oils yield equal or greater amounts, but tung, poppy, and walnut oils, and such seed oils as maize and cottonseed, yield almost no hexabromide, according to the figures compiled by Lewkowitsch never over 2 per cent.

Lewkowitsch recommends † that the test be applied to the mixed fatty acids rather than to the oil itself. In the separation of the acids care must be taken to avoid oxidation by exposure to air. The mixture of fatty acids from linseed oil yields 30 to 42 per cent. of hexabromide melting to a clear liquid at 175° to 180°, whereas the corresponding products from fish, liver, and blubber oils do not melt at this temperature but become darker and are completely

* *Analyst*, 1898, **23**, 310.

† Oils, Fats, and Waxes (3d Ed.), p. 459.

blackened at about 200°. Lewkowitsch states that this test is capable of showing the presence of 10 per cent. of fish oil in linseed.

OILS ALTERED BY AGE OR OXIDATION.

It has been assumed in discussing the analytical "constants" that the oils under examination are fresh or have been kept under such conditions as to prevent any material alteration. Age alone probably has no appreciable effect upon the analytical properties of commercially pure fatty oils, but such oils when kept for a long time in contact with air, for example, in partially filled or loosely stoppered vessels, take up atmospheric oxygen and gradually become considerably altered in those properties which are commonly regarded as "constants." This atmospheric oxidation naturally takes place much more rapidly with drying than with non-drying or semi-drying oils, and in open vessels than in those in which the oil is exposed to only a limited amount of air. It is probable that oils which have been thus altered are of much more frequent occurrence in commerce than has been supposed.

The influence of such oxidation upon the more important analytical properties is to increase the specific gravity, index of refraction, and temperature reaction with sulphuric acid, and to decrease the iodine number, the specific refractive power * and, in the case of olive oil, the viscosity of the soap solution. The acidity of the oil may increase at the same time but this change does not always occur.

The following results were obtained upon oils intentionally exposed to the air:

| Oil. | Iodine Number. | Sp. Gr $\frac{15.5°}{15.5°}$. | Index of Refraction at 15.5°. | Specific Refractive Power. | Specific Temperature Reaction. |
|---|---|---|---|---|---|
| Olive oil before exposure. | 83.8 | 0.9165 | 1.4712 | 0.5141 | 100 |
| Same after exposure. | 77.3 | 0.9240 | 1.4722 | 0.5100 | 127* |
| Lard oil before exposure. | 73.3 | 0.917 | 1.4697 | 0.5122 | 106 |
| Same after exposure. | 56.2 | 0.943 | 1.4724 | 0.5010 | 141 |
| Cottonseed before exposure. | 105.2 | 0.923 | 1.4737 | 0.5132 | 171 |
| Same after exposure. | 90.2 | 0.939 | 1.4779 | 0.5090 | 217* |
| Linseed before exposure. | 177.1 | 0.934 | 1.4835 | 0.5177 | |
| Same after exposure. | 136.9 | 0.969 | 1.4886 | 0.5042 | |

* These numbers were determined earlier than the other results on the exposed samples and therefore do not show the full effect of the change.

* Calculated by Landolt's formula $\frac{N-1}{D}$, *i. e.*, $\frac{\text{Index of refraction} - 1}{\text{Specific gravity}}$. (*Ber. deut. chem. Ges.*, 1882, 15, 1031.)

Many other oils have been tested with similar results. It is evident that oils thus altered are very likely to be misjudged, especially if only one or two quantitative determinations are made. Thus if only the specific gravity and temperature reaction of the olive oil had been determined the results would have been interpreted as indicating the presence of some seed oil. The iodine number of the linseed oil taken alone would indicate extensive adulteration with some oil of lower drying power. The results emphasize the importance of determining the specific gravity and the iodine number in all cases and show the advantage of determining the temperature reaction not as a substitute for the iodine number but for comparison with it. For a fuller discussion of this subject with the results obtained upon a number of other oils the reader is referred to two papers in the *Journal of the American Chemical Society* (July, 1903, and May, 1905).

As the result of all of this work it appears that the increase in specific gravity and the decrease in iodine number are almost exactly proportional to each other in non-drying and semi-drying oils, so that in examining an altered oil belonging to either of these classes the original iodine number can be estimated by adding 0.8 to the number found on the exposed sample for each increase of 0.001 in the specific gravity. When the original specific gravity is not known the calculation must be based upon the average specific gravity for oil of the species under examination. The error in this assumption can hardly be sufficient to affect the interpretation of the results.

The iodine numbers of exposed samples of linseed and fish oils cannot be corrected accurately by the rule given for semi-drying and non-drying oils, the number thus obtained being always too low.

Commercial "blown" oils show greatly increased specific gravities and decreased iodine numbers; the changes appear to bear much the same relation to each other as in the oils which have been altered by exposure.

## EXAMINATION OF AN UNKNOWN OIL.

In the examination of an unknown oil the specific gravity and the iodine and saponification numbers should be determined, and the appearance, odor, and taste compared with those of typical oils of known purity. The results thus obtained usually suffice to

locate the sample as one of a small group of oils, after which any special tests available for the detection of individual members of the group can be applied. The tests described in this chapter taken in connection with the quantitative determinations mentioned enable the analyst, in the majority of cases, to identify the oil or, if a mixture, the principal constituent.

If the saponification number indicates that only fatty oil is present, but the relation of the specific gravity to the iodine number does not correspond to that ordinarily found in any pure oil, the determination of the specific temperature reaction and the acidity will usually show whether the discrepancy is to be attributed to oxidation or adulteration.

The relative commercial value will of course determine what oils can profitably be used as adulterants. Prices vary greatly in different markets, as well as with the degree to which the oils are refined, and are also likely to fluctuate from year to year so that no fixed order of commercial value can be given.

The orders of commercial value given by Gill and by Lewkowitsch show considerable variation, which doubtless is due largely to the differences between American and English markets. In each of the lists the highest priced oils are given first.

Gill.—Almond, castor, sesame, neatsfoot, rape, olive, sperm, whale, peanut (arachis), linseed, tallow, lard, fish, cottonseed, mineral, rosin oil.

Lewkowitsch.—Almond, sperm, olive, neatsfoot, lard, cod liver, arctic sperm, arachis, poppy, sesame, seal, rape, castor, cottonseed, maize, linseed, whale, fish, mineral, rosin oil.

In the examination of mixtures containing other than fatty oils, it may be necessary to separate the mixed fatty acids and examine this mixture in order to identify the fatty oils originally present. The "constants" of the fixed fatty acids of various oils, as well as of many oils and fats not mentioned in this work, are conveniently tabulated in Lewkowitsch's Laboratory Companion to the Fat and Oil Industries.

## ADDITIONAL REFERENCES.

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Tolman and Munson: *Ibid.*, 1903, **25**, 954.

Mastbaum: *Chem. Rev. Fett und Harz Ind.*, 1904, **11**, 39, 64; *Ztschr. Unters. Nahr.-Genussm.*, 1904, **8**, 433.

### Arachis (Peanut) Oil.

Sadtler: *Amer. Journ. Pharm.*, 1897, **69**, 490; *Analyst*, 1897, **22**, 284.

Archbutt: *Journ. Soc. Chem. Ind.*, 1898, **17**, 1124.

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Bellier: *Bull. Soc. Chim.*, 1902, [3], **23**, 358.

Wijs: *Ztschr. Unters. Nahr.-Genussm.*, 1903, **6**, 692.

### Cottonseed Oil.

Oilar: *Amer. Chem. Journ.*, 1900, **24**, 355.

Weems and Grettenberg: *Proc. Iowa Acad. Sci.*, 1901; *Ztschr. Unters. Nahr.-Genussm.*, 1902, **5**, 465.

Fulmer: *Journ. Amer. Chem. Soc.*, 1902, **24**, 1148.

Wijs: *Ztschr. Unters. Nahr.-Genussm.*, 1903, **6**, 692.

Milliau: *Compt. rend.*, 1904, **139**, 807.

Fischer and Peyau: *Ztschr. Unters. Nahr.-Genussm.*, 1905, **9**, 81.

Emmett and Grindley: *Journ. Amer. Chem. Soc.*, 1905, **27**, 263.

### Sesame Oil.

Bellier: *Ann. Chim. Analyt.*, 1899, **4**, 217; *Analyst*, 1900, **25**, 50.

Bömer: *Ztschr. Unters. Nahr.-Genussm.*, 1899, **2**, 705.

Wijs: *Ibid.*, 1902, **5**, 1150.

Utz: *Chem. Rev. Fett und Harz Ind.*, 1902, **2**, 177; Abs. *Ztschr. Unters. Nahr.-Genussm.*, 1903, **6**, 621.

Kreis: *Chem. Ztg.*, 1903, **27**, 1030.

Lehnkering: *Ztschr. öffentl. Chem.*, 1903, **9**, 436; *Analyst*, 1904, **29**, 96.

### Maize Oil.

Hopkins: *Journ. Amer. Chem. Soc.*, 1898, **20**, 948.

Williams: *Analyst*, 1900, **25**, 146.

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POPPYSEED OIL.

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OXIDIZED OILS.

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Thompson and Ballantyne: *Ibid.*, 1892, **11**, 506.

Fahrion: *Ztschr. angew. Chem.*, 1898, 781.

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## CHAPTER XI

### Butter.

Butter is officially defined* as 'the product made by gathering in any manner the fat of fresh or ripened cream into a mass which also contains a small portion of the other milk constituents, with or without salt.' Standard butter contains not less than 82.5 per cent. of butter fat, having a Reichert-Meissl number not less than 24 and a specific gravity not less than 0.905 at 40°/40°. By acts of Congress approved August 2, 1886, and May 9, 1902, butter may also contain additional coloring matter.

Butter may fail to meet the requirements of the official standard either because of a deficiency in the percentage of fat or because of the presence of foreign fat or butter fat of abnormal character, though the sample may contain nothing which can be regarded as unwholesome. Butter analysis therefore includes (1) the examination of the whole butter, (2) the examination of the butter fat.

This chapter will be devoted mainly to the methods of examining butter fat but for convenience the determination of water, fat, curd, and ash will be described first. The point at which tests for preservatives can conveniently be made will be indicated, but the detection of foreign colors will not be considered as these are not regarded as adulterants.

#### DETERMINATION OF WATER, FAT, CURD, AND ASH.

Especial care must be taken in sampling butter since the water, salt, and curd are often unevenly distributed and an attempt to mix by stirring is apt to result in squeezing out drops of brine. If a large quantity is to be sampled a butter trier should be used, and the portions thus drawn united until a working sample of about 500 grams is obtained.

Melt the sample at the lowest possible temperature in a wide-mouthed glass-stoppered bottle, shake violently to ensure a homo-

* Circular No. 13, Office of the Secretary, U. S. Dept. Agriculture.

geneous mixture and continue the shaking while cooling the sample until it is sufficiently solidified to prevent any separation of water and fat.

Thoroughly clean and dry a lipped dish or beaker having a flat bottom of at least 20 square centimeters. Weigh the dish, introduce 1.5 to 2 grams of butter and re-weigh quickly to avoid evaporation. Dry to constant weight in a boiling water oven. The loss is *water*. Treat the dry residue with petroleum ether or benzine, transfer it to a weighed Gooch crucible having a felt of ignited asbestos and wash with the solvent until all fat is removed. Dry the crucible and residue to constant weight in a water oven or an air bath not above 110°. The material dissolved by the petroleum ether or benzine is *fat*. Burn the *curd* at a temperature below a red heat and weigh the crucible containing the *ash*.

*Notes.*— The method given is essentially that of the Association of Official Agricultural Chemists.* If preferred the butter can be dried on clean dry sand or asbestos. When the latter is not used it is important that the butter form only a very thin layer on the bottom of the dish or beaker; otherwise the water sinks into the melted fat and is only very slowly expelled at the temperature of the boiling water oven. For convenience in transferring and washing the residue with petroleum ether the latter should be used in a small wash bottle having a ground glass stopper. Instead of drying in a dish and transferring the residue to a crucible, the butter may be weighed directly in a crucible two-thirds filled with fibrous asbestos, dried to constant weight and then extracted.† A device for facilitating this drying by passing a current of air through the heated crucible has recently been described by Bird.‡

Great care must be taken to burn the curd at the lowest temperature possible in order to avoid loss of chlorine (see Chapter I.). Any small amount of milk sugar which the butter might contain would be counted as curd in this analysis. The percentage of salt can be found by determining chlorine in the ash, or by repeatedly washing the butter with hot water in a separatory funnel and titrating the combined washings with a standard solution of silver nitrate. Good butter usually contains 10 to 14 per cent. water, 84 to 87 per cent. fat, 0.5 to 1.5 per cent. curd, 2.0 to

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* Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

† Richards and Woodman: Air, Water, and Food (2d Ed.), p. 201.

‡ *Journ. Amer. Chem. Soc.*, 1905, 27, 818.

4.0 per cent. ash if salted; if unsalted, 0.25 to 0.5 per cent. The water content of butter has often been limited by legal or trade standards to 16 per cent. This is considerably above the present average for creamery butter, as shown by an investigation made by the United States Department of Agriculture in 1902.* Of 800 samples from 400 creameries in 18 states, the average water content was 11.78 per cent.; 85 per cent. of the samples contained between 10 and 14 per cent.; the extreme limits were 7.20 and 17.62 per cent. The amount of salt added to butter varies greatly with the demands of different markets, but over 5 per cent. would be excessive unless the butter were intended for export to a tropical country. An excessive amount of curd indicates careless manufacture or fraudulent increase of weight and is likely to injure the keeping qualities of the butter.

## EXAMINATION OF BUTTER FAT.

### PREPARATION.

Melt 100 grams or more of the butter and allow it to stand at 45° to 55° until the water and salt settle to the bottom.† Pour off the melted fat by decantation and filter it through a dry paper in a funnel heated by a water jacket or supported in a drying oven kept at about 60°. The filtered fat, which must be free from turbidity, is received in a wide-mouthed bottle and kept stoppered in a cool place until analyzed.

### REICHERT-MEISSL OR REICHERT-WOLLNY NUMBER.‡

This number is a comparative measure of the proportion of volatile acids, and is the most important basis for deciding the purity of butter fat. Often the presence or absence of foreign fat in butter, or the proportion of butter fat in oleomargarine, is inferred from this number alone. In the interest of uniformity of results official chem-

* Circular No. 39, Bureau of Animal Industry; *Analyst*, 1903, 28, 184.

† This water solution can be tested for preservatives, of which boric acid and borax are most likely to be found in butter. For methods see the chapter on milk analysis, or for more detailed directions Leach's Food Inspection and Analysis, or Lewkowitsch's Oils, Fats, and Waxes.

‡ Reichert: *Ztschr. anal. Chem.*, 1879, 18, 69.

Meissl: *Dingler's Polytech. Journ.*, 1879, 233, 229.

Wollny: *Milch Ztg.*, 1887, 16, 609; *Analyst*, 1887, 12, 203, 235; 1888, 13, 8, 38.

These papers are reprinted in Ephraim's Original Arbeiten über Analyse der Nahrungsmittel.

ists have sought to describe the method in such detail as to eliminate variations due to manipulation.

*Reagents.* — 1. Caustic soda solution, made by dissolving sodium hydroxide (nearly free from carbonate) in an equal weight of distilled water.

2. Alcohol, 92 to 95 per cent., containing no appreciable amount of volatile acid, either free or combined.

3. Dilute sulphuric acid, made by mixing pure concentrated sulphuric acid with four times its volume of water.

4. An accurately standardized approximately tenth-normal solution of barium (or sodium) hydroxide.

5. A 1 per cent. solution of phenolphthalein in alcohol.

*Determination.* — Thoroughly clean and dry a flask of 250 to 300 c.c. capacity. Weigh the flask, thoroughly mix the melted fat, introduce 5.6 to 5.8 c.c. measured at about 50°, allow the flask and fat to cool for 15 to 20 minutes and re-weigh (or, if convenient, weigh exactly 5 grams of fat into the flask). Add 10 c.c. of the alcohol and 2 c.c. of the caustic soda solution, attach the flask to a reflux condenser and boil on a water bath or electric heater for at least half an hour to ensure complete saponification.*

Evaporate the alcohol by heating the flask in a steam bath, shaking occasionally to avoid danger of loss from frothing and to facilitate the removal of the alcohol. Add 132 c.c. of recently boiled distilled water, warm at 60° to 70° until the soap is completely dissolved, add 8 c.c. of the dilute sulphuric acid and a few pieces of pumice stone, re-stopper the flask or connect it with a condenser and warm without boiling until the fatty acids separate as a clear layer. Distil through a glass condenser, collecting the distillate in a flask accurately graduated at 110 c.c. The distillation should be so regulated that 110 c.c. will be collected in from 28 to 32 minutes. Mix the distillate, filter through dry paper, and titrate 100 c.c. of the filtrate, using 0.5 c.c. of the phenolphthalein solution as indicator, until the red color remains apparently unchanged for 2 minutes. Increase the burette reading by one tenth (on account of the 10 c.c. of distillate not titrated), and calculate the number of cubic centimeters of tenth-normal alkali which would have been required if exactly five grams of fat were taken for the determination. This is the Reichert-Meissl number.

*The saponification can also be accomplished by heating in a closed flask, using either aqueous or alcoholic alkali, or by means of the glycerol-soda solution proposed by Leffmann and Beam: *Analyst*, 1891, 16, 153.

*Notes.*—The number thus found does not represent the total volatile acids present. The yield is fairly uniform if the given conditions of dilution and distillation are maintained. Wollny submitted this method to an exhaustive examination and pointed out the following sources of error: (1) absorption of carbon dioxide during saponification, (2) formation of esters during saponification, (3) formation of esters during distillation, (4) coherence of fatty acids during distillation resulting in holding back some of the volatile acid, (5) variations in the proportion of volatile acid carried over, due to differences in size and shape of distillation apparatus. In order to avoid discrepancies from these and other causes he published an elaborately detailed system of manipulation and precautions. It has been shown that Wollny greatly overestimated the probable errors of the method as previously carried out and that some of his precautions are unnecessary, but as in the main they tend toward greater uniformity of results, they have been adopted with slight modifications by the Association of Official Agricultural Chemists, whose methods * are usually accepted as standard in the United States and should be followed exactly in any determination which is likely to be made the basis of legal action.

In Great Britain, a joint committee representing the Government Laboratory and the Society of Public Analysts has adopted the method essentially as described above with the following specifications for the apparatus to be employed: † Flask used for saponification and distillation; capacity, 300 c.c.; length of neck, 7 to 8 cm.; width of neck, 2 cm. The flask is connected with the condenser by means of a bent glass tube 7 mm. wide, so placed that the bend is 15 cm. above the top of the cork. At a distance of 5 cm. above the cork is a bulb 5 cm. in diameter. The flask is supported on a circular piece of asbestos 12 cm. in diameter, having a hole 5 cm. in diameter in the center, so that the bottom of the flask is heated by a free flame during the distillation. The British committee further prescribed that blank determinations be made and the volume of alkali found necessary to neutralize the distillate (which volume should not exceed 0.3 c.c.) be deducted in calculating the results of each determination. The number so obtained is called the Reichert-Wollny number.

The Reichert-Meissl or Reichert-Wollny number of butter fat is

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* Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

† *Analyst*, 1900, 25, 309.

usually between 24 and 34; that of cocoanut fat, between 6 and 8; of other edible fats and oils, usually less than 1.

### Specific Gravity.

The specific gravity of butter fat has often been determined either at 100° or at 37.8° (100° F.). The standard recently established for the United States prescribes a minimum specific gravity at 40°, water at the same temperature being taken as unity. Either a specific gravity flask or an Ostwald pyknometer can be used conveniently for the determination, the pyknometer being filled and adjusted while surrounded by water kept at the required temperature, then removed from the water bath, wiped dry on the outside, allowed to cool to the temperature of the balance and weighed.

### Saponification Number.

This is determined as described in Chapter IX. Since the normal saponification numbers of butter fat are only about 15 per cent. in excess of those of the fats commonly used as adulterants, the determination in order to be of much value must be very accurately made.

### Insoluble Fatty Acids — Hehner Number.

*Reagents.* — 1. The alcoholic potash solution used in the determination of the saponification number.

2. Alcohol, about 95 per cent. by volume, which leaves no appreciable residue upon evaporation.

*Determination.* — Saponify 4 grams of butter fat with 50 c.c. of the alcoholic potash solution, evaporate to a paste to expel alcohol, dissolve the soap in about 400 c.c. of hot water in a weighed beaker, add hydrochloric acid in excess of the amount required to neutralize the potash used, and heat nearly to boiling with occasional stirring until the fatty acids have collected in a clear layer on the surface. Cool thoroughly, pour the solution through a filter and wash the cake with cold water without removing it from the beaker. Stir up the fatty acids in the beaker with another portion of hot water (200 to 300 c.c.), cool thoroughly, filter, and wash again. Repeat this treatment three times. After a final thorough washing with cold water, put the beaker containing the fatty acids beneath the funnel and dissolve any fatty acids which the filter may contain by washing with hot 95 per cent. alcohol, allowing the washings to run into the beaker. Evaporate off the alcohol and

dry the beaker containing the fatty acids to constant weight in a boiling water oven.

*Notes.* — This is the modification of Hehner's method adopted by the Association of Official Agricultural Chemists * and by the chemists of the Government Laboratory, London.† The original method ‡ which is still largely used involves washing the melted fatty acids with hot water on a paper filter. The results thus obtained are usually 1 to 2 per cent. lower than those by the official method. In the hot filtration method there is danger of washing some of the melted fatty acid through the paper.

### Iodine Number.

The determination of the iodine number has been fully described in Chapter IX. As butter fat absorbs only 26 to 38 per cent. of its weight of iodine, a gram of sample can be used for each determination. According to Patrick § butter fat shows iodine numbers about 1 unit higher by the Hanus than by the Hübl method.

### Melting Point — Wiley's Method.||

*Apparatus and Reagents.* — 1. An accurate thermometer reading to 0.1 degree.

2. A tall beaker nearly filled with water and arranged to be heated gradually with constant stirring from bottom to top.

3. A wide test tube suspended in the water in the beaker and nearly filled with water and alcohol as follows: Half fill the tube with hot recently boiled distilled water, then pour a nearly equal volume of hot recently boiled alcohol into the tube, carefully floating the alcohol on the water with as little mixing of the liquids as possible.

*Determination.* — Allow a drop of the melted butter fat to fall upon a smooth piece of ice floating in recently boiled distilled water. A thin disc of fat about 1 cm. in diameter should be obtained. Remove the disc from the ice by forcing the latter below the water when the fat will come to the surface whence it is

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* Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

† Thorpe: *Journ. Chem. Soc.*, 1904, 85, 248.

‡ Hehner and Angell. Butter, its Composition and Adulterations. London, 1874. Hehner: *Ztschr. anal. Chem.*, 1877, 16, 145. Ephraim, *loc. cit.*

§ U. S. Dept. Agriculture, Bur. Chem., Bul. 81, p. 49.

|| Wiley's Agricultural Analysis, Vol. III.; Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

removed by means of a steel spatula or knife blade and dropped into the tube containing the water and alcohol. The disc sinks to the point where the density of the alcohol-water mixture is equal to its own. It must not touch the side of the tube. Suspend the thermometer so that the bulb hangs in the tube exactly level with the disc of fat. Gradually heat the beaker, keeping the water well stirred. After the disc begins to shrivel, indicating that the temperature is within a few degrees of the melting point, the heat must be applied very carefully. The temperature at which the fat becomes a sphere is taken as the melting point. Repeat the determination twice, heating the bath at such a rate that 8 to 10 minutes are required to raise the temperature through the last 2 degrees. The second and third determinations should agree within 0.2 degree.

*Notes.*— The special advantage of this method is that it avoids the discrepancies caused by the adherence of the melting fat to solid surfaces, which in most other methods makes it difficult to determine the exact point of fusion. It is important to secure a very thin disc of fat for the determination, to avoid all adherence of air bubbles, and to secure uniform heating of the thermometer bulb and the disc by occasionally swaying the former around the latter as the temperature approaches the melting point. By using hot recently boiled water and alcohol in preparing the test tube for the determination the danger of air bubbles is avoided.

## Additional Determinations.

The *index of refraction* and *Crismer's test* * based upon the critical temperature of solution in alcohol are considerably used in the examination of butter fat. They are especially useful where rapid "sorting tests" are required, as in food inspection laboratories where only the suspected samples are submitted to complete examination.

The *phytosteryl acetate test* † is occasionally employed as a means of detecting the presence of vegetable fat, but requires too much

* Crismer: *Bull. Assoc. belge. Chim.*, 1895, **9**, 71; 1896, **10**, 312; Abs. *Analyst*, 1895, **20**, 209; 1897, **22**, 71. See also—Weiss: *Pharm. Ztg.*, 41, 268; *Chem. Centrbl.*, 1896, I., 1212. Asboth: *Chem. Ztg.*, 1896, **20**, 685. Browne: *Journ. Amer. Chem. Soc.*, 1899, **21**, 990. Lewkowitsch: Oils, Fats, and Waxes (3d Ed.), 214, 861.

† Bömer: *Ztschr. Unters. Nahr.-Genussm.*, 1901, **4**, 865, 1070; 1902, **5**, 1018. Juckenack and Pasternack: *Ibid.*, 1904, **7**, 193. Lewkowitsch: *l. c.*, 373.

time and skill for ordinary use and is likely to give misleading results in the hands of an inexperienced person. Cocoanut oil is the only vegetable fat which could be used in any considerable quantity without being shown by the Reichert-Meissl number, and the presence of cocoanut oil is best shown by comparison of the Reichert-Meissl and saponification numbers or determination of the *insoluble volatile acids* as described later.

*Acidity* of butter fat is sometimes determined and interpreted as a measure of the rancidity, although the odor and taste which cause a butter to be regarded as rancid are more largely due to aldehydes and other decomposition products than to free fatty acids. On the assumption that acidity can serve as a measure of rancidity the term *degree of rancidity* is sometimes used as synonomous with degree of acidity, *i. e.*, to show the number of c.c. of normal alkali required to neutralize the free acid in 100 grams of fat. One "degree" is therefore equivalent to an acid number of 0.56 or to 0.28 per cent. of free oleic acid.

## Composition of Butter Fat.

Browne analyzed the mixture of fatty acids from a sample having a rather low iodine number (29.28) with the following results:—*

| Acids. | Percentage of Acid in Fat. | Corresponding Percentage of Triglyceride. |
|---|---|---|
| Oleic | 32.50 | 33.95 |
| Dioxystearic | 1.00 | 1.04 |
| Stearic | 1.83 | 1.91 |
| Palmitic | 38.61 | 40.51 |
| Myristic | 9.89 | 10.44 |
| Lauric | 2.57 | 2.73 |
| Capric | 0.32 | 0.34 |
| Caprylic | 0.49 | 0.53 |
| Caproic | 2.09 | 2.32 |
| Butyric | 5.45 | 6.23 |
| Total | 94.75 | 100.00 |

This calculation neglects the unsaponifiable matter, which according to Browne amounts to only about 0.1 per cent.

The composition of butter fat is, however, quite variable as will be seen from the range in analytical properties.

*Journ. Amer. Chem. Soc., 1899, 21, 823.

VARIATIONS AND RELATIONS OF ANALYTICAL PROPERTIES.

The Reichert-Meissl or Reichert-Wollny number is much the most important of the data obtained in the examination of butter fat. The proportion of volatile acids tends to decrease as the period of lactation advances. The estimated normal range for the other important properties has been given in the table at the end of Chapter IX.

Any of these properties may be influenced by the feeding or health of the animal and occasionally vary much beyond the usually accepted "normal" limits as is shown by the following data collected by Browne.*

| | General Limits. | Extreme Limits. |
|---|---|---|
| Reichert-Meissl number......... | 20–33 | 11.2[Morse]–41[Nilson] |
| Saponification number.......... | 220–236 | 216[Samelson]–245[Fischer] |
| Iodine number.................. | 26–38 | 19.5[Moore]–49.57 [Farnsteiner and Karsch] |

The results of analyses of 357 authentic samples of butter fat collected from various parts of Great Britain and examined at the Government Laboratory, London, have been arranged by Thorpe † according to the Reichert-Wollny numbers and averaged by groups to show the relations between the principal physical and chemical properties of pure butter fat.

The following table shows the averages for each group of samples. The first line, for example, gives the average Reichert-Wollny number for all samples in which this number lay between

RELATION OF PHYSICAL AND CHEMICAL PROPERTIES OF BUTTER FAT (THORPE).

| Number of Samples. | Reichert-Wollny Number. | Specific Gravity $\frac{37.8^{\circ}}{37.8^{\circ}}$ | Butyro-Refractometer Reading at 45°. | Saponification Number. | Insoluble Fatty Acids Per Cent. | Mean Molecular Weight of Insoluble Acids. |
|---|---|---|---|---|---|---|
| 7 | 22.5 | 0.9101 | 42.0 | 219.9 | 90.1 | 266.9 |
| 17 | 23.5 | 0.9104 | 41.5 | 221.6 | 89.7 | 265.5 |
| 15 | 24.5 | 0.9108 | 41.5 | 223.5 | 89.4 | 265.0 |
| 27 | 25.5 | 0.9110 | 41.3 | 223.6 | 89.3 | 264.2 |
| 37 | 26.5 | 0.9113 | 41.0 | 225.6 | 88.9 | 261.9 |
| 51 | 27.5 | 0.9114 | 40.6 | 227.0 | 88.7 | 261.7 |
| 78 | 28.8 | 0.9118 | 40.1 | 228.6 | 88.4 | 260.9 |
| 56 | 29.5 | 0.9120 | 40.1 | 230.2 | 88.3 | 259.6 |
| 41 | 30.5 | 0.9123 | 39.9 | 231.7 | 87.9 | 260.1 |
| 18 | 31.3 | 0.9125 | 39.7 | 232.5 | 87.9 | 258.0 |
| 10 | 32.6 | 0.9130 | 39.4 | 232.8 | 87.7 | 257.8 |

* *Journ. Amer. Chem. Soc.*, 1899, **21**, 632.

† *Journ. Chem. Soc.*, 1904, **85**, 248.

22.00 and 22.99, with the average of the same samples for each of the other determinations. The second line shows the averages for all samples having Reichert-Wollny numbers from 23.00 to 23.99, etc.

In order to show to what extent increase of volatile acids takes place at the expense of oleic acid, the iodine numbers of 50 of the above samples were determined. Arranging these in groups of 20 and 30 respectively, according to the Reichert-Wollny values, the following average figures were obtained:—

| | Reichert-Wollny Number. | Iodine Number. | Oleic Acid Per Cent. | Insoluble Acid Per Cent. | Mean Molecular Weight of Insoluble Acids. |
|---|---|---|---|---|---|
| First group, | 24.2 | 40.0 | 44.4 | 89.6 | 264.6 |
| Second group, | 30.8 | 32.4 | 36.0 | 88.1 | 259.8 |

## Detection of Oleomargarine.

Butter substitutes or "artificial butters," unless sold under special names indicating their origin, are collectively termed "oleomargarine" in America or "margarine" in England. The oleomargarine made in America * consists chiefly of refined lard, "oleo oil" (the

* The materials used in the manufacture of oleomargarine in the United States during the fiscal year ending June 30, 1899 (Senate Document No. 168, 57th Congress, 1st Session) were as follows:

| Material. | Quantity. | Percentage of the whole. | Value per pound. | Total value. |
|---|---|---|---|---|
| | *Pounds.* | | *Cents.* | |
| Neutral lard | 31,297,251 | 34.27 | 8 | $2,503,780.08 |
| Oleo oil | 24,491,769 | 26.82 | 9 | 2,144,917.69 |
| Cotton-seed oil | 4,357,514 | 4.77 | 6 | 522,025.08 |
| "Butter oil" | 4,342,904 | 4.76 | 6 | 260,520.00 |
| Sesame oil | 486,310 | .53 | 10 | 4,863.10 |
| Coloring matter | 148,970 | .16 | 20 | 29,296.00 |
| Sugar | 110,164 | .12 | 4 | 4,406.50 |
| Glycerin | 8,963 | .01 | 10 | 896.30 |
| Stearin | 5,890 | .007 | 8 | 459.60 |
| Glucose | 2,550 | .003 | 3 | 76.50 |
| Milk | 14,200,576 | 15.55 | 1 | 142,005.76 |
| Salt | 6,773,670 | 7.42 | 1 | 67,726.70 |
| Butter | 1,568,319 | 1.72 | 20 | 313,663.80 |
| Cream | 3,527,410 | 3.86 | 5 | 176,370.50 |
| Total | 91,322,260 | 100 | ........ | 6,171,007.61 |

"Butter oil" is commonly stated to be a special brand of cottonseed oil; but the high Federal tax laid upon artificially colored oleomargarine by the law of May 9, 1902, practically prohibits the use of coloring matters employed before that date and has led to the introduction of "butter oils" containing palm oil which is naturally highly colored (Crampton and Simons: *Journ. Amer. Chem. Soc.*, 1905, 27, 270).

soft part of beef fat), and cottonseed oil, often mixed with a small amount of butter and almost always churned with milk or cream. Palm oil and sesame oil are known to be used to some extent and other semi-drying and non-drying oils are probably utilized in some factories.

Hence in comparison with any of the common constituents of oleomargarine, butter fat is characterized by its high proportion of soluble volatile acids, together with a high percentage of lauric, myristic, and palmitic as compared with oleic and stearic acids. The presence of oleomargarine in butter fat, therefore, lowers the Reichert-Meissl and saponification numbers and the specific gravity, while it raises the percentage of insoluble acids and either the melting point or the iodine number or both.

Several European governments require that sesame oil be added to oleomargarine in order to facilitate its detection when mixed with, or substituted for, butter. Similarly the addition of butter to oleomargarine is sometimes forbidden or restricted in order to prevent the production of mixtures too closely resembling genuine butter. The essential features of the oleomargarine laws of the principal countries are given in Lewkowitsch's Oils, Fats, and Waxes.

### Detection of Cocoanut Fat.

Since cocoanut fat consists largely of glycerides of saturated acids of low molecular weight, it could be added in considerable quantity to butter fat of average composition without causing the latter to vary beyond the normal limits in any of the important analytical properties. A comparison of the Reichert-Meissl and saponification numbers, however, would lead to the detection of this adulteration since the former number is higher and the latter lower in butter than in cocoanut fat. In pure butter fat the value of the factor [Saponification number — (200 + Reichert-Meissl number)] varies from 3.4 to — 4.1; in pure cocoanut fat it varies from 47 to 50.7.*

Another method of showing the presence of cocoanut oil is to determine the volume of tenth-normal alkali required to neutralize the insoluble volatile acids from 5 grams of fat. Under the conditions described by Polenske † the results thus obtained are approx-

* Juckenack and Posternack: *Ztschr. Unters. Nahr.-Genussm.*, 1904, 7, 193.

† Polenske: *Ibid.*, 1904, 7, 273. See also — Lewkowitsch's Oils, Fats, and Waxes (3d Ed.), 853. Muntz and Coudon: *Ann. d. Inst. Agron.*, 1904; *Analyst*, 1905, 30, 155. Hesse: *Milchwirthschaftl. Centrbl.*, 1905, 1, 13; *Chem. Centrbl.*, 1905, I., 566.

imately quantitative, the percentage of insoluble volatile acids (mainly lauric acid) being much higher in cocoanut fat than in butter.

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# CHAPTER XII.

## Soaps and Lubricants.

Soaps, glycerol, and lubricants are the most important technical products of the fats and oils. Glycerol having been considered under the head of alcohols, methods for the examination of soaps and lubricants will be given in this chapter. Any compound of a mineral base with a fatty or resin acid may be called a soap. Since only the soaps of the alkalies are readily soluble in water, they alone are employed as detergents but insoluble soaps are frequently used as constituents of lubricating mixtures.

### ANALYSIS OF COMMERCIAL SOAP.

The determinations required in the examination of a commercial soap depend largely upon the purpose for which it is intended. The scheme outlined below is adaptable to almost all cases as it can be easily extended to include additional determinations or shortened by omitting such steps as are unnecessary when only a partial analysis is required.

A cake of soap exposed to the air dries rapidly at the surface, forming a horny layer which to some extent prevents the evaporation of water from the interior. In sampling such a soap it is important to remember this variation in water content of different parts of the same cake. If the sample is to represent the individual cake as it existed at the time of commencing the analysis, a number of sections through the entire cake should be taken either by slicing or by means of a cork borer or a cheese or butter sampler. Often the purpose of the analysis is to show the composition of the sample as originally sold, in which case the dried surface should be rejected and the sample taken from the center of the cake. The sample for analysis should be reduced quickly to fine shavings and kept in a tightly stoppered bottle.

### DETAILS OF DETERMINATIONS INDICATED IN THE SCHEME.

#### *I. Determination of Water.*

If the soap is very hard and dry it may be reduced to fine shavings and dried on a watch-glass, heating first for some time at

## Outline Scheme of Analysis.*

First Portion. Dry 2 to 5 grams of soap (I.)† and introduce into Soxhlet extractor, either in a thimble or supported by a firm plug of cotton, and extract with petroleum ether.

<table>
<tr>
<td rowspan="3">Solution. Evaporate the solvent; dry and weigh the residue as unsaponified fat and unsaponifiable matter. (II.)</td>
<td colspan="3">Residue. Allow petroleum ether to evaporate and exhaust thoroughly with boiling water.</td>
</tr>
<tr>
<td colspan="2">Solution. Decompose with a considerable excess of standard sulphuric (or hydrochloric) acid, separate aqueous solution from fatty layer. (III.)</td>
<td rowspan="2">Residue. Dry and weigh as insoluble matter.<br>Ignite and weigh as insoluble mineral matter. (X.)</td>
</tr>
<tr>
<td>Solution. Add methyl orange and titrate for total alkali. Test for chlorides (or sulphates), sugar, and glycerol. (IV.)</td>
<td>Fatty Layer. Dry and weigh as fatty and resin acids. (V.)</td>
</tr>
</table>

Second Portion. Exhaust 2 to 5 grams of the fresh sample with carefully neutralized alcohol. (VI.)

<table>
<tr>
<td rowspan="3">Solution. Add phenolphthalein and titrate for free caustic alkali or free fatty acid. (VII.)</td>
<td colspan="3">Residue. Dry and weigh. (VIII.) Exhaust thoroughly with boiling water.</td>
</tr>
<tr>
<td colspan="2">Solution. Divide into aliquot parts.</td>
<td rowspan="2">Residue. May be examined instead of residue from first portion or used for additional tests or determinations. (X.)</td>
</tr>
<tr>
<td>Add methyl orange and titrate for alkali carbonate (and borate, silicate, or aluminate if present). (IX.)</td>
<td>Test for (and if necessary determine) sulphate, borate, silicate, and aluminate. (IX.)</td>
</tr>
</table>

40° to 60° and then finishing at 105° to 110°. Very few soaps can be completely freed from moisture in this way but some others may be sufficiently dried for the ether extraction while the moisture determination is made on a separate sample as follows: Dissolve about two grams of soap in the minimum quantity of hot strong alcohol and evaporate on clean dry sand. Finish the drying at 110° with frequent stirring, a small rod having been weighed with the dish. *For approximate determination of moisture* Allen recommends Smith's method: Heat 5 to 10 grams of finely divided soap in a large porcelain crucible on a sand bath over a small Bunsen flame. Stir continually with a small glass rod (weighed

* Allen: Commercial Organic Analysis, Vol. II., Part I.

† Numbers in parenthesis refer to sections which follow.

with the crucible) having a roughened end to facilitate breaking any lumps of soap which may be formed. Continue heating until there is no more evidence of water being expelled, then test by removing the burner and placing a cold glass at once over the crucible. If no moisture condenses on the glass the soap is considered dry. With practice the drying can be finished in 20 to 30 minutes. Any burning of the soap is readily detected by the odor. The results are said to be reliable to 0.25 per cent.

*II. Petroleum Ether Extract.*

Transfer the thoroughly dried soap to a paper "fat extraction thimble," plug tightly with fat-free cotton and treat in a Soxhlet extractor, heated on a safety water bath or electric heater, with petroleum ether of nearly constant boiling point. Regulate the heating so that the extract fills to the syphoning point in ten or fifteen minutes and continue the extraction for four to six hours. Disconnect the extraction apparatus (observing that no free flame is near) as the solvent flows through the syphon to the flask; remove the thimble, reconnect the apparatus, and recover the solvent by placing a wide short test-tube in the space previously occupied by the thimble, or by allowing the solvent to collect in this space and removing it before it reaches the top of the syphon. Having expelled nearly all of the petroleum ether, heat the flask containing the extract in a boiling water oven to constant weight.

The petroleum ether extract of a commercially pure soap may contain unsaponified fat (or free fatty acids, which are now largely used in soapmaking) as well as the "unsaponifiable matter" of the original soap grease. Hydrocarbons, phenols, and other substances soluble in petroleum ether may also be found in mixed and "medicated" soaps. Allen * gives directions for the systematic examination of this extract including the quantitative determination of phenol if present.

*III. Liberation of Fatty and Resin Acids.*

Decompose the water solution of the soap by adding a considerable excess (allow 5 to 7 c.c. of normal acid for each gram of dry soap) of normal or half-normal sulphuric, nitric, or hydrochloric acid. Add the entire amount of acid at once, boil, stir thoroughly for some minutes, and keep the solution hot until the fatty

* Commercial Organic Analysis, Vol. II., Pt. I. (3d Ed.), pp. 279–284.

acids collect at the surface, leaving the water solution nearly clear, then complete the separation as in the determination of insoluble acids in butter.

It is convenient to liberate the fatty acids in a tared beaker in which they can afterward be dried and weighed (V.). If the fatty acids are liquid at ordinary temperature or form a cake too soft to be handled conveniently, a known weight of dry bleached beeswax or stearic acid may be added to the hot solution. The fatty acids become incorporated with the wax and on cooling a firm cake is obtained.

*IV. Solution Separated from Fatty Acids.*

This solution contains, in the form of sulphate (or chloride if hydrochloric acid be used to decompose the soap), all the alkali originally present as soap, as carbonate (silicate or borate), or as hydroxide. On titrating this solution with alkali, using methyl orange as indicator, the amount of acid found to have been neutralized gives a measure of the total alkali of the soap. Unless potash is known to be present, this total alkali is usually calculated as sodium oxide. The solution also contains any chlorides or other soluble salts, soluble fatty acids, glycerol, sugar, etc., which the soap may have contained. After titration the solution can be diluted to a known volume and separate portions taken for qualitative tests and quantitative determinations.

When sulphuric acid has been used to liberate the fatty acids, *chlorides* can be determined in a portion of the neutralized solution. If it is desired to determine both chloride and sulphate in this solution the soap can be decomposed by means of standard nitric acid. It is usually more convenient to test for sulphates in the residue from the alcohol extraction as described below.

*Soluble fatty acids* will be found in this solution if the liberated acids of cocoanut or palmnut oil soaps are washed with hot water, as is often recommended. When the fatty acids are separated cold and washed with cold water only, the amount dissolved can usually be neglected without appreciable error.

*Sugar*, if present, is detected in a part of this solution. After further treatment with acid to ensure complete hydrolysis, the invert-sugar is determined either volumetrically or gravimetrically (Chapter V.). Sugar may also be determined by means of the polariscope using a separate portion of the sample and precipitating the fatty acids as insoluble barium soaps.*

* [illegible]

In the absence of sugar and other interfering substances, *glycerol* can be determined by treating a portion of the neutralized solution directly with sulphuric acid and standard dichromate as described in Chapter III. (Hehner's method). Since the results thus found are often too high, because of the presence of organic impurities, Lewkowitsch recommends * the following method: Decompose the water solution of the soap with sulphuric acid, separate the fatty acids, neutralize the filtrate with barium carbonate, evaporate to a syrup, and extract with a mixture of 3 parts 95 per cent. alcohol and 1 part ether. The glycerol thus obtained can be determined by the acetin method after complete removal of alcohol, but results sufficiently accurate for ordinary work can be obtained by drying, weighing, and burning the glycerol as in the analysis of fermented liquors. The method for glycerol in wines (Chapter III.) can also be used to separate glycerol from sugar in analyzing a soap containing both of these.

### *V. Mixed Fatty and Resin Acids.*

The mixture of acids liberated as already described (III.) is dried to constant weight in a boiling water oven in a weighed flat bottomed dish or beaker as in the determination of the insoluble acids in butter fat. The weight having been found, test a portion for resin acid by the Liebermann-Storch reaction as described under drying oils (Chapter X.). In the absence of resin acids it may be possible to show the nature of the fat from which the soap was made, by examining the mixed fatty acids according to the methods used in identifying fats and oils as described in Chapters IX. and X., consulting special works such as those of Lewkowitsch or Benedikt-Ulzer for the "constants" which cannot be inferred from the properties of the corresponding fats. *The separation of fatty and resin acids* is best accomplished by *Twitchell's method* based upon the difference of behavior of these acids when exposed in alcoholic solution to the action of hydrochloric acid. By this treatment fatty acids are converted to ethyl esters while resin acids remain practically unchanged. The method is carried out by Lewkowitsch † as follows: Weigh 2 to 3 grams of the mixed acids in a flask, dissolve in 10 times their volume of absolute alcohol, immerse the flask in cold water, and pass a current of dry

* Oils, Fats, and Waxes (3d Ed.), p. 1081.

† Oils, Fats, and Waxes (3d Ed.), p. 394.

hydrochloric acid gas through the solution for an hour; then dilute the contents of the flask (which will have separated into two layers) with 5 times its volume of water and boil until the aqueous solution has become clear. Transfer the contents of the flask to a separating funnel by means of 50 cc. of petroleum ether (boiling below 80°); shake, allow to separate, draw off the acid solution, and wash the petroleum ether layer once with water. After the latter has separated completely and been removed, add a solution of 0.5 gram of potassium hydroxide and 5 c.c. of alcohol in 50 c.c. of water; shake, and allow to separate. The ethyl esters remain dissolved in the petroleum ether while the resin acids are extracted by the dilute alkaline solution forming soaps. Draw off the soap solution, wash the petroleum ether solution again with dilute alkali, unite the alkaline solutions; liberate the resin acids by means of hydrochloric acid, collect, dry, and weigh them as in the determination of liberated fatty acids.

The resin acids can be titrated after washing free from hydrochloric acid, instead of being separated and weighed. The volumetric method is more rapid than the gravimetric but necessitates the assumption of a combining weight (346) for the resin acids, which is liable to considerable inaccuracy. According to Lewkowitsch, the results by the volumetric method are likely to be too high; those by the gravimetric method too low.

### *VI. Extraction with Alcohol.*

Dry soap can be extracted with 95 per cent. alcohol; for wet soap stronger alcohol should be used so that after taking up the moisture of the sample it will still be too strong to dissolve an appreciable amount of carbonate. The alcohol to be used must first be very carefully neutralized, using phenolphthalein as indicator. In this neutralization there is danger of adding an excess of alkali unless it is remembered that the full pink color of the indicator will not appear in alcohol of this strength. If difficulty is experienced in detecting the neutral point a small amount of the alcohol can be removed and mixed with an equal volume of boiling water to bring out the color of the indicator.

While the extraction of the soap with alcohol is often carried out in open vessels, filtering and washing in the ordinary way, it is usually more satisfactory to use the Soxhlet extractor. The soap can be put in a paper thimble as in the petroleum ether extraction

or between plugs of cotton in a glass tube with perforated bottom. In the latter case the progress of the extraction can be watched without disconnecting the apparatus. When the extraction is complete the residue should be in powder form. If distinct pieces remain these may contain soap which has been protected from the action of the alcohol by the formation of a layer of insoluble salts. In this case remove and crush the residue, replace, and extract again.

*VII. Free Caustic Alkali or Fatty Acid.*

To the alcoholic extract add a few drops of neutralized phenolphthalein solution. If the solution reacts alkaline, titrate with tenth-normal acid for caustic alkali; if acid, titrate with tenth-normal alkali for free acid. For a further discussion of this extract including a rapid method for the partial analysis of soaps, see Allen, *l. c.*, pp. 291–294.

*VIII. Residue Insoluble in Alcohol.*

It is advisable to dry and weigh this residue so that the percentage of impurities not actually determined can be found by difference. A microscopic examination may also be of use in determining the subsequent treatment. Starch and gelatine if present could be separated from carbonate, borate, and sulphate by dissolving the latter salts in cold water; but silicate would probably be incompletely dissolved, and it is therefore better as a rule to extract with hot water and to use separate portions of the soap, if necessary, for the determination of starch and gelatin. In such a case extract the soap with alcohol and in the residue determine starch as described in Chapter VII., or determine nitrogen by the Kjeldahl method and calculate the corresponding amount of gelatin, taking the nitrogen content of the latter as 17.9 per cent.*

*IX. Carbonate, Silicate, Borate, and Aluminate.*

Add to the water extract from the residue insoluble in alcohol an excess of standard acid and boil to ensure decomposition of the silicate. If it is important to distinguish quantitatively between carbonate and the other alkaline salts present, the carbonic acid given off during this boiling can be collected and weighed. Add methyl orange as indicator and titrate with standard alkali to determine the total amount of alkali which was present as carbonate, silicate, borate, and aluminate.

To a portion of the solution add hydrochloric acid in excess, evaporate to small volume and test for boric acid by means of turmeric paper; when dry, heat at 110°, take up with dilute hydrochloric acid, filter out and determine silica, if present.

Other portions of the solution or the filtrate from silica can be used for the detection and determination of sulphates, aluminates, etc.

*X. Insoluble Matter.*

This residue should be dried to constant weight at 100°, a portion examined microscopically and the remainder ignited and weighed. If over one per cent. of insoluble mineral matter is found it should be analyzed. Among the substances which may be found in this residue are oatmeal, bran, sawdust, clay, chalk, steatite, infusorial earth, pumice, sand, mineral pigments, etc.

### Calculation and Interpretation of Results.

In the case of hard soap, the results of the partial analysis usually required may be reported as follows:

Water; unsaponified fat and unsaponifiable matter; fatty and resin anhydrides (97 per cent. of the weight of free acids); sodium oxide combined as soap; sodium hydroxide; sodium carbonate; insoluble organic matter; insoluble mineral matter. It is well to report also the total alkali in terms of sodium oxide.

The purpose for which a soap is intended must be known before an opinion as to its quality can safely be formed. In most cases the percentage of alkali combined as soap is the best measure of the amount of actual soap in the material, but for special purposes the presence or absence of other constituents is often of greater importance.

Toilet soaps should contain as little free alkali (either caustic or carbonate) as possible. Alder-Wright divided toilet soaps into three classes according to the proportion of free alkali to alkali combined as soap. The first class included those soaps which contained less than 2.5 per cent. as much free as combined alkali; the second, those in which the percentage was 2.5 to 7.5; the third, those containing over 7.5 per cent. as much free as combined alkali. In judging the quality of toilet soaps it is also important to consider the proportions and nature of all foreign matter, the amount of water, the hardness of the soap and in some cases the origin must be sought by an examination of the fatty

acids. The more expensive "transparent" toilet soaps may contain alcohol or glycerine; in cheaper grades a similar appearance is obtained by the addition of sugar.

Household soaps are made from cheaper and softer fats than those used for toilet soap. Alkali in the form of carbonate, silicate, or borate is not objectionable unless present in excessive amount. No appreciable amount of sugar or glycerol is likely to be present. Scouring soaps often contain large amounts of pulverized quartz, infusorial earth, etc., and are sometimes strongly alkaline with sodium carbonate or hydroxide.

The qualities of soaps for special and technical purposes are discussed in several of the following works.

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## EXAMINATION OF LUBRICATING GREASES.

Lubricating greases are usually mixtures of soaps with fats, hydrocarbons, rosin, or tar, containing water and sometimes large amounts of mineral matter. On melting, the grease often separates into a soap solution and an oily layer. The soaps used in making such lubricants may contain salts of sodium, calcium, or heavy metals with either fatty or resin acids. Some greases consisting essentially of fats and hydrocarbons melt at the temperatures to which they are subjected in use and may therefore be examined in the melted state by the methods used for lubricating oils. For most greases, however, it is necessary to adapt the analytical method to the nature of the mixture to be examined in each case, since the composition of these greases is too variable to allow the use of any fixed system of examination. It may often be necessary to resort to a combination of the methods used in the analysis of soaps, fats, and lubricating oils. For detailed information on the composition and testing of lubricating greases the reader is referred to the reference books cited at the end of this chapter and to a review by Conradson: *Journ. Amer. Chem. Soc.*, 1904, **26**, 705–712.

## EXAMINATION OF LUBRICATING OILS.

A thorough examination of lubricating oil involves: (1) the determination of the nature of the oil and, if it is a mixture, the proportion of each constituent; (2) tests to determine the efficiency and safety of the oil as a lubricant with special reference to the conditions of temperature, pressure, etc., to which it will be subjected in use. Among the most important properties of lubricating oils which can be measured in the laboratory are viscosity, acidity, the temperature at which the oil solidifies, and the flashing and burning points. The usefulness of other determinations will depend upon the purposes for which the oil is intended.

DETERMINATION OF CONSTITUENTS.

Pure fatty and mineral oils are largely used as lubricants, both singly and mixed with each other in all proportions. Other substances are, however, often added to reduce the cost or to increase viscosity of the oil, among the most common being rosin oils and "gelatin oils" containing aluminum oleate or other soaps. A better but more expensive means of increasing viscosity is to use castor oil or a "blown" oil.

In beginning the examination of a lubricating oil, note carefully any color, odor, turbidity, or fluorescence which may aid in identifying the oil or detecting foreign substances The presence of soap is easily shown by igniting a portion of the oil, as refined fatty and mineral oils should not yield over 0.05 per cent. of ash. Rosin oil can be detected by the Liebermann-Storch reaction.

After making these preliminary observations the saponification number should be determined as this may show the sample to be a practically pure fatty or mineral oil. In saponifying mixtures consisting largely of heavy mineral oil there is difficulty in securing sufficient contact between the sample and the alcoholic potash solution even though petroleum ether or gasoline be added. In such cases a Soxhlet extractor can be placed between the flask and the reflux condenser.* The intermittent syphoning of the condensed solvent from the extractor into the saponification flask mixes the contents and facilitates saponification. In order to diminish the volume of solvent required and the interval between stirrings, the body of the extractor is filled with glass beads. Having found the saponification number (Chapter IX.), if the sample appears to be a mixture of fatty and mineral or rosin oil the proportions of saponifiable and unsaponifiable matter are found either by separating and weighing the latter or by estimating the former from the amount of fatty acids recovered from the soap solution after saponification.

*Determination of Unsaponifiable Oils.*

Weigh 2 to 10 grams of oil (depending upon the saponification number and the method to be followed), saponify by heating with alcoholic potash on a water bath,† evaporate off the alcohol,

* Conradson: *Journ. Amer. Chem. Soc.*, 1904, **26**, 672.

† See also the method involving cold saponification given by Fahrion: *Ztschr. angew. Chem.*, 1898, 267.

and separate the unsaponifiable matter by one of the following methods.

*Method of Immiscible Solvents.* — To the residue from the evaporation of alcohol add 75 c.c. of water, stir thoroughly, transfer to a separatory funnel, add about an equal volume of petroleum ether or washed ethyl ether, close the funnel, shake vigorously, and allow to stand over night or until the aqueous and ethereal solutions separate completely. Draw off the aqueous layer into another separatory funnel; wash it again with ether and the ethereal layer again with water; repeat if necessary. Finally unite the ether solutions in a weighed flask, distil off the ether, and dry the unsaponifiable oil to constant weight in a boiling water oven.

If desired, the fatty acids can be recovered from the aqueous soap solution by adding an excess of mineral acid and shaking with ether or by separating the fatty acids as in soap analysis.

The principal objection to the separation by immiscible solvents is that emulsions frequently form in the separating funnel which remain even on standing for a day or more. The addition of 1 to 2 c.c. of alcohol often helps to break the emulsion but if more alcohol is added it tends to carry soap into the ether layer. The separation of the solvents is also facilitated by chilling the funnel and twirling it gently or, if the apparatus is available, by whirling in a centrifuge. Petroleum ether dissolves less soap than ethyl ether and gives less troublesome emulsions but does not always extract the unsaponifiable matter completely.

*Extraction of the Dry Soap.* — To avoid difficulties of the preceding method A. C. Wright * recommends the following: Saponify 10 grams of oil using 5 grams of caustic potash; after evaporating off the alcohol, add 8 grams of sodium bicarbonate and 20 c.c. of pure methyl alcohol, stir well and evaporate, add 10 c.c. more of methyl alcohol and 25 grams of precipitated chalk, mix well, dry on a water bath and then for a few minutes at 110°. Transfer the thoroughly dried mixture quickly to a Soxhlet extractor and extract the unsaponifiable matter with petroleum ether. Dry the extract to constant weight in a boiling water oven and weigh.

The mixture of calcium carbonate and soap from which the unsaponifiable matter has been extracted can be treated with hydrochloric acid to dissolve the carbonate and liberate the fatty acids which can then be separated and examined further.

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* Analysis of Oils and Allied Substances, p. 111.

*Estimation and Identification of Fatty Oils.*

From the weight of fatty acid recovered as described above, the percentage of fatty oil can be calculated on the assumption that the oil yields 95 per cent. of insoluble fatty acids. The result thus found serves as a check upon the direct determination of unsaponifiable oil.

If only fatty and mineral oils are present and the percentage of the former is small, it can be estimated with sufficient accuracy for most purposes from the saponification number, since the fatty oils which are likely to be present in mixed lubricants do not vary greatly in their saponification numbers. See table at end of Chapter IX. If the fatty oil is identified the average number for that species of oil should be used in estimating the percentage.

If the lubricant consists entirely of fatty oil with a known small amount of inert unsaponifiable matter, the usual methods for the identification of fatty oils can be employed. Otherwise the identification is based upon the examination of the separated fatty acids.

## VISCOSITY.

*Apparatus and Methods.*

The viscosity of an oil can be determined either by measuring the resistance which it offers to the movement of a submerged solid, or by observing the rate at which it flows through an aperture under given conditions of temperature and pressure. Torsion viscosimeters such as that of Doolittle * depend upon the first principle but those depending upon the measurement of the rate of flow are much more generally used. Viscosimeters of this kind are made in a great variety of forms for descriptions of which the reference books at the end of the chapter can be consulted. Among the viscosimeters most commonly used in the United States are those of Redwood, Engler, and Saybolt.

*Redwood's viscosimeter* consists of a cylinder about 4.7 cm. in diameter and 8.7 cm. high, having in the center of the bottom a cup-shaped agate jet which can be closed by means of a spherical plug. Inside the cylinder is a small fixed bracket of thick bent wire with an upturned point to indicate the height to which the oil should extend at the beginning of the test. The apparatus is jacketed and provided with a closed side tube and a revolving

* *Journ. Amer. Chem. Soc.*, 1893, 15, 174.

stirrer so that determinations can be made at high temperatures if desired. The apparatus is intended to deliver 50 c.c. of water at 15.5° in 25.5 seconds, but as the rate of flow is influenced by many conditions it must be determined by each observer for his own apparatus and method of working. The instrument must be very carefully cleaned and dried before and after using as any dust (or scratches caused by careless cleaning) in the agate jet will alter the rate of flow.

To use the apparatus at room temperature place it on a level support, insert the plug, and fill with the liquid to be tested until the surface comes exactly to the upturned point already mentioned. Place beneath the outlet a narrow-necked flask graduated at 50 c.c., open the jet by lifting the ball valve, and observe the time required for 50 c.c. to flow into the receiving flask. A stop watch is recommended but is not absolutely essential. Repeat the determination as often as is necessary to obtain an average result having a probable error of less than 2 per cent. Whatever method is adopted for expressing the results,* the report should always show the actual time of flow for the oil and for water and the temperature at which the test was made.

In working at any temperature other than that of the room, fill the outer jacket of the viscosimeter with water, or for temperatures above 95° with mineral oil. This is first brought to the required temperature, the oil to be tested (previously brought to the same temperature) is then introduced and the receiving flask is placed in a bath of liquid at the temperature at which the test is to be made. At high temperatures great care must be taken in heating and stirring as a variation of 1° or less will sometimes make an appreciable difference in the time of flow.

The Redwood viscosimeter is generally regarded as standard in Great Britain having been adopted† by the War Department, the Scottish Mineral Oil Association, and the principal railway companies. Engler's viscosimeter occupies a similar position in Germany and the "normal apparatus" of this form is now manufactured under the joint control of the Charlottenburg Mechanisch-technische Versuchsanstalt and the Karlsruhe Chemisch-technische Versuchsanstalt. For description and illustration of this apparatus

* Results are sometimes compared with standard rape oil requiring 535 seconds at 15.5°. Other methods of expressing viscosity are given by different writers; see references at the end of the chapter.

†Lewkowitsch: Oils, Fats, and Waxes (3d Ed.), p. 199.

see Lewkowitsch's Oils, Fats, and Waxes (3d Ed.), pp. 202–204. Directions for use are furnished with each apparatus. For a description of Saybolt's viscosimeter which is made in three forms for testing different types of oils consult Gill's Oil Analysis (3d Ed.), pp. 24–28.

*Significance of Results.*

Since the object of lubricating with oil is to separate the moving surfaces by a fluid layer, it is important that the oil have sufficient viscosity or "body" to stay in place and keep the moving surfaces apart under the maximum pressure to which they will be subjected. The greater the pressure the more viscous the oil should be, but any viscosity beyond that which is necessary to keep the oil in place means an increase of fluid friction and consequent loss of power. The viscosity of the oil is therefore the most direct indication of its suitability as a lubricant under given conditions. For several reasons, however, the viscosity alone is not a conclusive measure of the lubricating power. The adhesion to solid surfaces which prevents the displacement of the oil by pressure is not always directly proportional to the true viscosity or internal friction. Oils vary greatly in the rate of change of viscosity with increasing temperature and pressure. The viscosity as measured by the rate of flow depends not only upon the internal friction of the oil, but also to some extent upon its adhesion to the sides of the outlet and upon the specific gravity. Hence it is not to be assumed that any two oils having the same viscosity can be used interchangeably as lubricants. In order to duplicate an oil which has been found satisfactory in use, the kind of oil, the specific gravity, and the viscosity shall all be specified.

Viscosity is especially important in dealing with mineral oils because of the ease with which they can be varied in this respect, while any particular kind of fatty oil varies only within comparatively narrow limits. For a full theoretical discussion of viscosity and lubrication the work of Archbutt and Deeley should be consulted. The reader must also be referred to this and other books and articles given below for discussion of the many practical considerations affecting the selection of lubricating oils.

## ACIDITY.

Weigh accurately 5 to 10 grams of oil in a 250 c.c. Erlenmeyer flask, add 50 c.c. of neutralized 85 per cent. alcohol containing

phenolphthalein as indicator and titrate with standard sodium or potassium hydroxide shaking vigorously after each addition until a permanent pink color is obtained. It is often necessary to allow the flask to stand after shaking until the oil separates from the alcohol solution before observing the color of the latter.*

To test for free mineral acid, shake 10–15 grams of oil with 100 c.c. of warm water in a separatory funnel, allow to separate, draw off the water, filter through wet paper, cool, and add methyl orange. If mineral acid is found, shake the oil remaining in the funnel repeatedly with small portions of hot water until all mineral acid is extracted, filter as before, add the filtrate to the first portion containing methyl orange and titrate very carefully with standard alkali. Concentrate the neutralized solution, test qualitatively to determine the nature of the acid and calculate the percentage. If the identification of the mineral acid is prevented by the presence of salts, calculate the mineral acidity as due to sulphuric acid. The acidity due to organic acids, or the total acidity if only this is determined, is usually calculated as percentage of oleic acid. As much as 15 per cent. of free oleic acid is sometimes allowed in lubricating oils. The best grades of lard oil do not contain over 1.5 per cent. Free mineral acids should be absent.

### COLD TEST AND CHILLING POINT.

The "cold test" indicates the temperature at which the sample is just sufficiently melted to flow; the "chilling point" that at which the oil begins to become turbid or to show flocks or scales of solid. In either case the temperature required will be influenced by details of manipulation, so that an arbitrary method must be followed to obtain strictly comparable results. The following directions follow the procedure of the Pennsylvania railroad laboratory.†

*Cold Test.* — Pour about 25 c.c. of oil into an ordinary sample bottle of about 100 c.c. capacity and introduce a short stout thermometer. Place the bottle in a freezing mixture until the sample

* For determining acidity in very dark colored fats the use as indicator of 10 c.c. of a 2 per cent. solution of "Alkali Blue II OLA" (Meister, Lucius, and Brunig) in 99 per cent. alcohol has been recommended by Freundlich: *Oesterr. Chem. Ztg.*, 1901, 4, 441; *Ztschr. Unter. Nahr.-Genussm.*, 1902, 5, 460.

† Motive Power Department Specifications, No. 75, Altoona, Pa., 1895. For the method and apparatus of the Chicago, Burlington, and Quincy Railroad laboratory see Stillman's Engineering Chemistry, 2d Ed., pp. 352–354.

is solid throughout. Remove the bottle and allow the solidified oil to soften, stirring and mixing by means of the thermometer until the mass will flow from one end of the bottle to the other. The reading of the thermometer at this point is the "cold test."

*Chilling Point.* — Usually it is only necessary to know whether the oil remains clear for a given number of minutes at a given temperature. Use the same bottle, amount of sample, and thermometer as for the cold test. Expose the liquid to cold, stirring with the thermometer and hold at the required temperature for the specified time (usually ten minutes). If the oil remains transparent and free from flocks or scales, it meets the requirement as to chilling test.

If it is required to find the chilling point the procedure is similar, but the liquid after remaining clear as described is exposed to a temperature 3° lower, allowed to stand with constant watching and occasional stirring with the thermometer until the oil is as cold as the bath, repeat this cooling until opacity or flocks or scales begin to show. The reading of the thermometer when this occurs shows the "chilling point."

For further information on the cold test or setting point see the works of Archbutt and Deeley, Gill, Holde, Lewkowitsch, and Stillman.

### Flashing and Burning Points.

For the most accurate results closed testers such as are used in examining illuminating oils should be employed, but as such accuracy is not required in testing lubricants it will usually be sufficient to carry out the test without the use of any special apparatus as follows: *

On a sand bath and partly imbedded in it place a porcelain dish about 6.5 cm. in diameter and 2.5 cm. deep. Fill the dish to about 0.6 cm. of the top with the oil to be tested and hang a thermometer so that the bulb is immersed in the oil at the center of the dish without touching the bottom. Heat by means of a Bunsen burner and as the flashing point is approached test at each third or fourth degree by passing the test flame slowly entirely across the dish about 1 cm. above the level of the oil and directly in front of the thermometer. Record the temperature at which the first flash is seen as the *flashing point.* Continue heat-

* Specifications of the Pennsylvania Railroad Motive Power Department, Altoona, Pa., 1902.

ing and testing in the same way until the liquid takes fire; note the temperature as the *burning point.* Remove the thermometer and blow out the flame or smother it by sliding a watch glass over the dish.

The test must be made in a place protected from drafts. The test flame should be about 0.5 cm. long and is conveniently obtained from a narrow glass jet connected with the ordinary gas tubing. The flow of gas is easily regulated to give a flame of the required size. The heating of the sandbath should be so regulated that the temperature of the oil rises 6° to 8° per minute, the rise being more rapid the higher the flashing point. The test flame can then be applied every half minute.

Any variation from the conditions given, either in the size and form of dish, the rate of heating and testing, or the manner of applying the test flame may cause an appreciable discrepancy in the result.

### ADDITIONAL DETERMINATIONS.

Additional tests and determinations are frequently required to show the suitability of the lubricant for the particular use intended. *Friction tests* on oil testing machines especially designed for the work are sometimes of great importance. A full discussion of such mechanical methods of testing will be found in Archbutt and Deeley's Lubrication and Lubricants.

*Loss by evaporation and tendency to "gum"* are tested by heating a small amount of oil on a watch glass for several hours at the highest temperature to which it is likely to be subjected in use. The oil must not become sticky and the loss of weight should usually be less than one per cent. *Suspended matter* which may be invisible in a dark oil is detected by diluting the sample with gasoline or petroleum ether. *Antiflourescents* added to destroy the fluorescence or "bloom" of mineral oils can often be detected by boiling 1 c.c. of the oil with 3 c.c. of a 10 per cent. solution of potassium hydroxide in alcohol. A red color indicates nitro-naphthalene or nitro-benzene, which are the principal antifluorescents used. According to Holde* asphalt pitch is used to thicken cylinder oil and can be approximately determined as follows: Dissolve 5 grams in 125 c.c. of ether at 15°, add, drop by drop with constant shaking, 62.5 c.c. of 96 per cent. alcohol; after standing 5

*Mitt. Kgl. Techn. Versuchsanstalt, Berlin*, 1902, 20, 253; *Ztschr. Unters. Nahr.-Genussm.*, 1903, 6, 855.

hours at 15°, filter, wash with a mixture of alcohol and ether (1 : 2 by volume) until nothing more than a trace of pitch-like substance is removed. Dissolve the residue in benzol, evaporate, dry one half hour at 105°, and weigh.

For other tests and determinations and fuller discussions of most of those here given the reader is referred to the works given for reference below.

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## CHAPTER XIII.

### Proteids and Cereals.

PROTEIDS AND RELATED COMPOUNDS.

The proteids are very complex and usually amorphous compounds, differing in composition and properties, but all of high molecular weight and unknown chemical structure. They all contain carbon, hydrogen, nitrogen, sulphur, and oxygen; some also contain phosphorous and a comparatively small number iron. The limits of ultimate composition of the well-known simple proteids are as follows: *

| *Carbon.* | *Hydrogen.* | *Nitrogen.* | *Sulphur* | *Oxygen.* |
|---|---|---|---|---|
| 51.3–55.0 | 6.7–7.3 | 15.5–19.3 | 0.3–2.2 | 20.8–23.5 per cent. |

Proteids differ in their solubilities in water, salt solutions, and alcohol but are all insoluble in ether, chloroform, carbon tetrachloride, carbon bisulphide, benzene, and petroleum ether.

The proteid compounds are of the greatest importance in physiology and food chemistry, being the fundamental organic constituents of the living substance of both animal and vegetable cells, and the only group of organic compounds which is strictly essential to the nutrition of all animals. The proteids and closely related compounds have therefore been studied and classified largely from the physiological standpoint. While differences exist both in classification and terminology, the following is believed to represent the present usage of the majority of English-speaking physiologists and physiological chemists.†

SIMPLE PROTEIDS.

*Albumins.*—Soluble in water and dilute salt solutions; precipitated by adding sufficient alcohol or by saturating the aqueous solution with ammonium sulphate. The water solutions are

* Ultimate analyses of a number of proteids have recently been compiled and discussed by Osborne: *Journ. Amer. Chem. Soc.*, 1902, 24, 160.

† Compare American Text-book of Physiology and reference books given at the end of the chapter.

coagulated by heating, usually at 70°–73° (Halliburton), but sometimes much lower, leucosin coagulating at 52° (Osborne).

*Globulins.* — Insoluble in pure water but soluble in dilute salt solutions. Precipitated by alcohol or by saturation with ammonium or magnesium sulphate; also precipitated from salt solutions on removing the salt by dialysis. Some of the vegetable globulins are distinctly crystalline and not coagulable by heating.

*Alcohol Soluble Proteids.** — These occur especially in the cereal grains. Gliadin, insoluble in water, dilute salt solution, or absolute alcohol, but soluble in 75 per cent. alcohol is the best-known member of this group.

*Albuminates.* — By the action of acid or alkali upon native or coagulated proteid the latter is converted into an acid- or an alkali-albumin or albuminate. Both acid and alkali albuminates are nearly insoluble in water and dilute salt solution, but usually dissolve readily in water containing a very small amount of acid or alkali and are precipitated from such solutions by neutralization at ordinary temperature. The albuminates are precipitated from nearly neutral solutions by the general precipitants for soluble proteids (see below); but a very minute amount of acid or alkali is sufficient to prevent the coagulation of albuminate by boiling. Acid albumin, also called *syntonin*, is formed by the acid of gastric juice as the first step toward peptic digestion.

*Proteoses and Peptones.* — These are products derived from other proteids by digestion or by simple hydrolysis. They are soluble in water and not coagulated by boiling their aqueous solutions. No sharp line can be drawn either between proteoses and peptones or between peptones and the simpler nitrogen compounds which result from prolonged digestion. As the terms are generally used, *peptones* may be considered the final products of digestion or hydrolysis which are still proteids as judged by the biuret reaction (see below) and are precipitable by tannin (perhaps not always completely) or by addition of strong alcohol. *Proteoses* (albumoses) are intermediate products between albuminates and peptones. In addition to the proteid reactions shown by peptones the proteoses are precipitated from aqueous solutions at ordinary temperatures by adding acetic acid and potassium ferrocyanide or by saturating the solution with zinc or ammonium sulphate.

* The classifications commonly given relating especially to animal proteids omit this group.

The term peptone was formerly applied to all digestion products not coagulated by boiling and is still popularly used in the same sense, the best commercial "peptones" consist largely of proteoses.

*Coagulated Proteids.*—This group includes proteids which have been coagulated by heating or by the action of reagents or enzymes. The nature of the changes which take place in coagulation is not known. The coagulated proteids are insoluble in water, alcohol, salt solutions, or very dilute acids. They are dissolved and converted into albuminates by acids and alkalies, especially on heating.

Some plant proteids such as the glutenin of wheat are insoluble in the neutral solvents but dissolve readily in very dilute alkali. These may be native insoluble proteids or they may have been coagulated by an enzyme in the plant.

## COMBINED PROTEIDS.

*Chromo-proteids.*—There are compounds of simple proteids with coloring matter usually containing iron. Hæmoglobin and its derivatives are the best-known examples.

*Glyco proteids.*—Glyco- or gluco-proteids are compounds of proteids with carbohydrates. They are practically insoluble in water but easily soluble in very weak alkalies. On boiling with dilute mineral acids they yield considerable amounts of reducing sugars. Mucins and mucoids belong to this group.

*Nucleo-proteids.*—These are compounds of proteids and nuclein or nucleic acid, an organic acid yielding phosphoric acid on decomposition. True nuclein also yields on decomposition one of the purin bases and is thus distinguished from paranuclein which it resembles in other respects. Nucleo-proteids are found especially in cell nuclei and are therefore particularly abundant in the highly nucleated cells of secreting organs such as the liver, pancreas, etc.

While all of the simple proteids are lævorotatory, the nucleo-proteids thus far studied* have shown dextro-rotation and the nucleic acid of the wheat embryo has been found to be strongly dextrorotatory.†

*Paranucleo-proteids or Nucleo-albumins.*—These compounds of proteids with paranuclein are often classified as a sub-group of the true nucleo-proteids which they resemble closely in composition. Recently, however, the purin bases have been extensively studied

*Gamgee and Jones: *Amer. Journ. Physiol.*, 1903, 8, 447.

†Osborne: *Ibid.*, 1903, 9, 69.

and shown to be of much physiological importance, and since paranucleins do not yield purin bodies on decomposition, the paranucleoproteids are now frequently classified as an independent group under the name of nucleo-albumins and are sometimes considered as simple proteids containing phosphorus. Casein and vitellin (the phosphorus-containing proteid of egg yolk) are examples of this group.

### Albuminoids (Albumoids, Proteoids, Gelatinoids).

This division includes several groups of compounds differing considerably from each other but all closely related to the true proteids included in the above groups and all characterized by their resistance to reagents. They are apparently formed from proteids in the body but cannot take the place of proteids in nutrition. Among the more important of the albuminoids are *collagens* which yield *gelatins* on boiling with water; *keratins* of skin, horn, hair, feathers, nails, etc.; *elastin* of connective tissue; *skeletins*, the nitrogenous compounds of the skeletal and related tissues of invertebrates, including the characteristic compounds of sponges and silk.

Gelatins (and collagens) are the only albuminoids which are likely to be met in connection with the true proteids in foods, etc., so that the other substances mentioned need not be considered in connection with the terminology and reactions given below.

### Other Nitrogen Compounds — Terminology.

Among the organic nitrogen compounds which occur with proteids in many animal or vegetable substances are the lecithins and related compounds, alkaloids, and the so-called "nitrogenous extractives" including amines, amids, and amino-acids. Ammonium salts and nitrates may occur in plant and animal tissues but usually only in minute amounts. They may, however, be added to food materials as preservatives. In most natural food products the total amount of these simpler nitrogen compounds is small as compared with that of proteids and it has become customary in food analysis to take the total nitrogen as a measure of the proteids present, so that the percentage of proteids as reported ordinarily means the percentage of nitrogen multiplied by 6.25, this factor being based on the assumption that proteids contain approximately 16 per cent. of nitrogen.

*Protein* is the term now commonly used to designate the results thus arbitrarily estimated from the nitrogen content, *proteids* being preferably reserved as a general name to cover the simple and combined proteids (and sometimes also gelatin) while the simpler nitrogen compounds are grouped together as *non-proteids*. Beyond this division into proteids and non-proteids the terminology of the nitrogen compounds of foods is in a very confused state. That recommended by the Association of American Agricultural Colleges and Experiment Stations and largely used by agricultural writers in this country is as follows:*

- Protein. Total nitrogen compounds.
  - *Proteids*
    - Albuminoids
      - Simple
        - Albumins
        - Globulins and allies
      - Modified
        - Derived
        - Compound
    - Collagens or gelatinoids
  - Non-proteids: Extractives; amids, amino acids, etc.

The most important point to be noted here is the use of the word "albuminoids." Formerly this was used synonymously with proteids to designate (1) simple and combined proteids and (2) gelatin and related compounds. As the differences between these two classes of compounds have become better known the majority of physiologists and physiological chemists have confined the term albuminoids to the latter class in order to distinguish these compounds from the true proteids, while in the agricultural terminology just quoted the compounds of the first class are called albuminoids. Some writers propose to discard the term "albuminoids" entirely, retaining "simple and combined proteids" and using for the related compounds some new term such as "proteoids" or "albumoids."

## General Reactions of Proteids.

### *Color Reactions.*

Among the most important color reactions are:—

1. *The xanthoproteic reaction.*—Proteids are colored yellow by nitric acid of 1.2 specific gravity or stronger. The color is intensified by heating and changes to orange or red on treatment

* U. S. Dept. Agriculture, Office of Experiment Stations, Bull. 65, p. 117; Armsby's *Principles of Animal Nutrition*, p. 7.

with an excess of ammonia. According to Halliburton * this reaction depends upon the presence of an oxybenzene nucleus.

2. *Millon's Reaction.* — On heating with Millon's reagent † (a solution of mercuric nitrate containing nitrous acid) proteid matter (including gelatin) gives a brick-red coloration which according to Nasse is also due to the presence of any oxybenzene nucleus in the proteid molecule. For detailed discussions of this reaction the papers of Vaubel ‡ and Nasse § should be consulted.

3. *Adamkiewicz' or Tryptophan reaction.*—When proteid is treated first with glacial acetic acid and then with concentrated sulphuric a violet color usually appears. Hopkins and Cole ‖ found that the reaction occurred only when the acetic acid contained traces of glyoxylic acid and that a better reaction is obtained by mixing the proteid with a little glyoxylic acid solution¶ and afterward adding concentrated sulphuric acid. The color then appears at the line of contact of the liquids. Gelatin does not give the reaction (Hammarsten). According to Osborne and Harris ** the vegetable proteids vary greatly in their reactions, some of them giving little if any violet color. This reaction is due to the presence of the tryptophan group in the proteid molecule, tryptophan being probably skatol-amido-acetic acid (Hopkins and Cole).

For other color reactions due to tryptophan see Cole: *Journ. Physiol.*, 1903, **30**, 311.

4. *Piotrowski or Biuret Reaction.*—On adding a very dilute solution of copper sulphate drop by drop to a proteid solution strongly alkaline with sodium or potassium hydroxide a rose-red to violet coloration appears. A more pronounced rose color is obtained with proteoses and peptones than with other proteids. To test for

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* Schäfer's Textbook of Physiology, I., 47.

† To prepare the reagent dissolve mercury in twice its weight of nitric acid, 1.42 sp. gr., and dilute the solution obtained with three times its volume of water. According to Nasse a better method is to use a solution of mercuric acetate containing a few milligrams of sodium or potassium nitrite, the solution having been recently acidulated with acetic acid.

‡ *Ztschr. angew. Chem.*, 1900, 1125.

§ *Arch. ges. Physiol.* (*Pflüger*), 1901, **83**, 361.

‖ *Proc. Royal Soc.*, 1901, **68**, 21; *Journ. Physiol.*, **27**, 418; **29**, 451.

¶ Prepared as follows: Place a saturated solution of oxalic acid in a tall cylinder, add lumps of sodium amalgam (about 60 grams per liter of solution), allow to stand as long as hydrogen is evolved then filter and dilute the solution with twice its volume of water.

** *Journ. Amer. Chem. Soc.*, 1903, **25**, 853.

peptones in a solution containing other proteids,* precipitate the latter by saturation with zinc sulphate, filter, to the filtrate add caustic soda until the zinc hydroxide first precipitated is completely dissolved, then add a few drops of a one per cent. solution of copper sulphate. This test is said to show as little as 1 part of peptone in 100,000 of solution. For other proteids it is less delicate. The reaction is given by many substances other than proteids; according to Schiff† by any compound containing two amino groups united either directly by their carbon atoms or by means of a third carbon or a nitrogen atom. Examples of these three classes of compounds are oxamid, malonamid, and biuret.

*To establish the presence of proteid* all of these tests should be applied since any one of them may be given by substances other than proteids. By combining color reactions with some of the precipitation reactions given below conclusive evidence of the presence of proteid can be obtained.

*Precipitation Reactions.*

*Coagulation by heating* and precipitation by *saturation with zinc or ammonium sulphate* have been mentioned in describing the different groups. It should be noted that the presence of other nitrogen compounds including urea, pyridine, and piperidine interferes with the coagulation of proteids.‡

*Nitric* or *metaphosphoric acid* precipitates most proteids other than peptones. *Potassium ferrocyanide* precipitates all true proteids other than peptones from solutions acidulated with acetic acid. Gelatin is not precipitated. *Copper, lead,* and other heavy metals form insoluble compounds with proteids. Basic lead acetate is much used to remove proteids from solutions to be examined in the polariscope and cupric hydroxide has been commonly employed for the separation of proteids from amids in vegetable foods. *Tannin* in the presence of salt precipitates proteids, including peptones, completely (Allen).§ *Phosphotungstic acid* in the cold precipitates proteids and simpler nitrogen compounds, especially the diamino-acids; in hot solutions small amounts of peptones and simpler compounds are not precipitated. *Bromine* added to saturation precipitates proteids including peptones and gelatin. According to Allen the precipitation is quantitative.

* Neumeister: *Ztschr. Biol.*, 1890, 26, 324.

† *Ber. deut. chem. Ges.*, 1896, 29, 298; Hammarsten-Mandel. p. 31.

‡ Spiro: *Ztschr. physiol. Chem.*, 1900, 30, 182.

§ The completeness of the precipitation of peptones is sometimes questioned.

As most of these precipitation reactions are also given by alkaloids, and some of them by amino-compounds, it is important in order to show the presence of proteids to precipitate with as many reagents as possible. The precipitates obtained by heat coagulation and by acetic acid and potassium ferrocyanide* should be tested for the color reactions described above. For other precipitation reactions see Halliburton: Schäfer's Textbook of Physiology, I., Chapter I., and Cohnheim: Chemie der Eiweisskörper. The completeness of precipitation by several of the reagents mentioned, and the interference of other substances are discussed in the papers cited at the end of the next section.

## Separation of Proteids from Simpler Nitrogen Compounds and from Each Other.

The separation of proteids from simpler nitrogen compounds is usually based upon the precipitation of the former by some reagent which will not precipitate the other nitrogen compounds present in the substance under examination. The choice of precipitant will therefore depend upon the nature of the substance in which proteid is to be determined. Cupric hydroxide has been most commonly used for vegetable, and tannin, phosphotungstic acid, or bromine for animal substances.

The methods of separating proteids from each other are based almost entirely upon differences in solubilities and coagulation or other precipitation reactions such as have been given in characterizing the groups already mentioned. As the reactions of the peptones approach those of some of the compounds of known structure, the separation of proteids from simpler compounds and from each other can best be studied as parts of the same problem. The methods available for these separations are so dependent upon the particular combination of compounds to be separated that any attempt to give detailed directions would be of less value than references to the original publications among the more important of which are the following: —

Stutzer: Untersuchungen ueber die quantitative Bestimmung des Proteinstickstoffes und die Trennung der Proteinstoffe von anderen in Pflanzen vorkommenden Stickstoffverbindungen, *Journ. f. Landwirthschaft*, 1880, 28, 103; 1881, 29, 473; 1886, 34, 151.

Schulze und Barbieri: Zur Bestimmung des Eiweisstoffe und

* Winternitz: *Ztschr. physiol. Chem.*, 1892, 16, 439.

der nichteiweissartigen Stickstoffverbindungen in der Pflanzen, *Landw. Vers. Stat.*, **26**, 218.

Teller: The Quantitative Separation of Wheat Proteids, Bul. 42, Ark. Agl. Expt. Sta., p. 81 (1896).

Allen and Searle: Improved Method of Determining Proteid and Gelatinoid Substances, *Analyst*, 1897, **22**, 258.

Baumann und Bömer: Ueber die Fallung der Albumosen durch Zinksulfat, *Ztschr. Unters. Nahr.-Genussm.*, 1898, **1**, 106.

Mallet: Analytical Methods for Distinguishing between the Nitrogen of Proteids and that of the Simpler Amids or Amino-acids, Bul. 54, Div. Chem., U. S. Dept. Agriculture; *Chem. News*, 1899, **80**, 117, 168, 179.

Vivian: A Comparison of Reagents for Milk Proteids, 16th Ann. Rpt. Wis. Agric'l. Expt. Sta., p. 179 (1899).

Barnstein: Ueber eine Modifikation des von Ritthausen vorgeschlagenen Verfahrens der Eiweissbestimmung, *Landw. Vers. Stat.*, 1900, **54**, 327; *Ztschr. Unters. Nahr.-Genussm.*, 1901, **4**, 688.

Schjerning: [A series of papers on the quantitative separation and precipitation of proteids], *Ztschr. anal. Chem.*, 1894, **33**, 263; 1895, **34**, 135; 1896, **35**, 285; 1897, **36**, 643; 1898, **37**, 73, 413; 1900, **39**, 545, 633.

Fraps and Bizzell: Methods of Determining Proteid Nitrogen in Vegetable Matter, *Journ. Amer. Chem. Soc.*, 1900, **22**, 709.

Hart: Ueber die Quantitative Bestimmung der Spaltungsprodukte von Eiweisskörpern, *Ztschr. physiol. Chem.*, 1901, **33**, 347.

Van Slyke and Hart: Methods for the Estimation of the Proteolytic Compounds contained in Cheese and Milk, Bul. 215, New York Agl. Expt. Sta., *Amer. Chem. Journ.*, 1903, **29**, 150.

Bigelow: Meat and Meat Products, U. S. Dept. Agriculture, Bur. Chem., Bul. 65, pp. 10, 17; Bul. 13, Part 10, p. 1396; Bul. 81, p. 104.

Grindley: A Study of the Nitrogenous Constituents of Meats, U. S. Dept. Agriculture, Bur. Chem., Bul. 81, p. 110, *Journ. Amer. Chem. Soc.*, 1904, **26**, 1086.

Chamberlain: Determination of Gliadin and Gutenin in Flour, U. S. Dept. Agriculture, Bur. Chem., Bul. 81, p. 118; Circular 20, p. 14.

Grindley and Emmett: The Chemistry of Flesh, *Journ. Amer. Chem. Soc.*, 1905, **27**, 658.

## CEREALS AND OTHER GRAINS — MILL PRODUCTS.

In the analysis of these products as of other foods and feeding stuffs of vegetable origin the constituents usually determined are moisture, fat, proteid, fiber, and ash. The remaining constituents, estimated by difference, constitute the so-called nitrogen-free extract composed mainly of carbohydrates. When little fiber is present its determination is frequently omitted, in which case fiber and nitrogen-free extract are reported together, usually as "carbohydrates by difference." When a further examination of any one of these groups of compounds is desired it can be made according to the methods already described. The fat obtained by extraction with ether can be studied as outlined in Chapters IX. to XI., the separation of the nitrogen compounds has been discussed in the preceding part of this chapter, and methods for the determination of the principal carbohydrates are given in Chapters V. to VII. The present outline will therefore be confined to the determinations first mentioned. Samples for analysis should be air-dried and ground to pass a sieve of one half millimeter mesh, though for very resistant substances a sieve having round holes of one millimeter diameter may be used.

### DETERMINATION OF MOISTURE AND FAT.

Dry 2 grams for 5 hours, or to constant weight, at the temperature of boiling water, if possible in a current of dry hydrogen or in vacuo. Consider the loss of weight as moisture. Extract the dried sample in a Soxhlet or continuous extractor, with anhydrous alcohol-free ether for sixteen hours. Dry the extract to constant weight in a boiling water oven. The ether extraction should be carried out at a distance from any free flame, the flask being heated by a safety water bath or, more conveniently, by an electric heater.

*Notes.* — The ether extract of vegetable substances often contains in addition to fat more or less of coloring matters and resinous substances, these being more readily soluble in ether containing fatty oils than in ether alone. In the cereal grains and especially in the milling products from which the outer layers of the grains have been separated the amount of such impurities is usually negligible. If the ether extract is made to percolate a layer of animal charcoal, practically pure fat is obtained.*

* Patterson: *Amer. Chem. Journ.*, 12, 261.

The reason for drying in a current of hydrogen rather than in air is that the oils of the cereal grains belong to the "semidrying" group and therefore absorb oxygen when exposed to air, especially at high temperature. This will of course increase the weight of the fat and make the apparent percentage of moisture too low. The partially oxidized oils are also apt to be incompletely extracted by ether. For a full discussion of the determination of water in foods and physiological preparations, see Benedict and Manning: *Amer. Journ. Physiol.*, 1905, 13, 309.

Fat may also fail of complete extraction, even when unchanged, by being occluded or mechanically enclosed in carbohydrate or proteid material which is impervious to the ether.

Although ether extracts may be evaporated at the temperature of boiling water without loss of fat, such loss has been found to occur in drying moist samples even in a current of hydrogen. That the loss in such cases is, in part at least, due to an actual volatilization of material may be shown by passing the current of hydrogen in which the sample is dried into strong sulphuric acid. This loss is probably due to the action of the escaping steam and may be practically avoided by drying at a lower temperature, preferably in a partial vacuum.

All three of the causes just mentioned tend toward a deficiency of fat in the analysis of cooked foods prepared from cereal products. Thus in a number of experiments on bread making * the fat found by analysis of the dried bread was less than half of that contained in the materials used and the iodine figure of the ether extract of the bread was only 60.4 as against 101.4 in that of the original flour showing that a very considerable oxidation had taken place even in that portion of the fat which was still soluble in ether.

*Berntrop's method* for the determination of fat in breadstuffs is as follows: † Mix 150 grams of fresh bread with 500 c.c. of water, add 100 c.c. of concentrated hydrochloric acid and boil for two hours connected with a reflex condenser.‡ Cool the resulting brown liquid to room temperature, filter with suction through a moistened fat-free paper, and wash with cold water. Dry the paper and residue for an hour at 100° to 110°, remove the residue as com-

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* Bul. 67, Office of Experiment Stations, U. S. Dept. Agriculture.

† *Ztschr. angew. Chem.*, 1902, 121.

‡ In treating meal or flour, heat for an hour in a waterbath and then boil for an hour with the reflux condenser attached.

pletely as possible from the filter paper and grind it with sand in a mortar. Cut up and add the filter paper, and transfer the dry mixture to a paper extraction thimble and treat with ether or petroleum ether in an extractor.

*Dormeyer's method*,* designed originally for the determination of fat in animal tissues has been adapted to vegetable foods by Beger.* From 3 to 5 grams of substance are mixed with 480 c.c. of water, 20 c.c. of 25 per cent. hydrochloric acid, and one gram of fat-free pepsin. The mixture is kept at 37° to 40° for twenty-four hours, filtered with suction through a paper supported on a porcelain plate and covered with asbestos, and both the filtrate and the residue extracted with ether.

## Determination of Nitrogen Compounds.

Determine total nitrogen as described in Chapter II. For the separation of proteid from non-protied nitrogen in cereals. Stutzer's cupric hydroxide method‡ has been most commonly used and is "official" for agricultural products. In examining wheat flour with reference to its baking qualities it is desirable to determine also the salt-soluble and the alcohol-soluble proteids using the methods recently adopted provisionally by the Official Agricultural Chemists.§

The extended investigations by Osborne and his associates || and by Ritthausen ¶ have shown that nearly all of the cereal proteids contain over 16 per cent. of nitrogen so that the results obtained by multiplying the nitrogen by 6.25 are too high. The factors now regarded as most nearly correct are; for wheat, rye, and barley, 5.70 **; for maize, oats, rice, and buckwheat, 6.00. The old factor 6.25 is, however, still frequently used for the sake of uniformity or for comparison with earlier work. In reporting results, therefore, the factor used should always be given.

* *Arch. f. ges. Physiol.* (*Pflüger*), 1895, 61, 341; 1896, 65, 90.

† *Chem. Ztg.*, 1902, 26, 112.

‡ Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

§ Circular 20, Bur. Chem., U. S. Dept. Agriculture.

|| Reports Conn. Agl. Expt. Station, 1890, *et seq.* Much of the work has also appeared in *Journ. Amer. Chem. Soc.*, *Amer. Chem. Journ.*, and *Amer. Journ. Physiol.*

¶ Summarized in *Landw. Vers. Stat.*, 1896, 47, 391.

** The factor 5.68 has recently been proposed for wheat flour.

### Determination of Crude Fiber.*

Extract 2 grams of the substance with ordinary ether or use the residue from the determination of the ether extract. To this residue, in a 500 c.c. flask, add 200 c.c. of boiling 1.25 per cent. sulphuric acid; connect the flask with an inverted condenser, the tube of which passes only a short distance beyond the rubber stopper into the flask. Boil at once, and continue the boiling for thirty minutes. A blast of air conducted into the flask may serve to reduce the frothing of the liquid. Filter, wash with boiling water till the washings are no longer acid; rinse the substance back into the same flask with 200 c.c. of a boiling 1.25 per cent. solution of sodium hydroxide, practically free from sodium carbonate; boil at once, and continue the boiling for thirty minutes in the same manner as directed above for the treatment with acid. Filter on a Gooch crucible, and wash with boiling water till the washings are neutral; dry at 110°; weigh; incinerate completely. The loss of weight is crude fiber.

The filter used for the first filtration may be linen, one of the forms of glass wool or asbestos filters, or any other form that secures clear and reasonably rapid filtration. The solutions of sulphuric acid and sodium hydroxide are to be made up of the specified strength, determined accurately by titration and not merely from specific gravity.

### Determination of Ash.

Char about 2 grams and burn to whiteness at the lowest possible red heat, preferably in a flat bottomed platinum dish in a muffle.

If considerable quantities of phosphates are present these may fuse over some of the carbon and render its combustion very slow. In such cases, extract the charred mass with a little hot acetic acid, set aside the solution till the char is burned, then evaporate it to dryness in the same dish and heat the residue to dull redness till the ash is white or nearly so. Samples containing added salt should be extracted with water before charring and the determination finished as just described. The precautions given in Chapter I. should always be observed.

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* Bul. 46, Bur. Chem., U. S. Dept. Agriculture. See also Thatcher: *Journ. Amer. Chem. Soc.*, 1902, 24, 1210; Browne: *Ibid.*, 1904, 25, 315.

### Additional Tests and Determinations.

For discussions of tests for foreign substances present as adulterants the references given below should be consulted. Such adulteration of cereal products is not common in America. The determinations most often required, in addition to those already given are such as to show the usefulness of the material for some particular purpose. The value of wheat flour for breadmaking depends largely upon the proportion of alcohol-soluble proteid (gliadin) which it contains, and upon the acidity. The gliadin is most abundant in the interior of the wheat kernel and therefore in the highest commercial grades of flour. Acidity is objectionable both as an indication of deterioration and because it acts upon the gliadin injuring the physical properties which are especially important in breadmaking.

To determine acidity, shake 10 grams of the dry sample with 100 c.c. of cold water, filter, and titrate an aliquot part with tenth-normal sodium or potassium hydroxide using phenolphthalein as indicator. In fine flour the acidity calculated as lactic acid should not exceed 0.10 per cent.

### Interpretation of Results.

*Official Definitions and Standards.**

1. *Grain* is the fully matured, clean, sound, air-dry seed of wheat, maize, rice, oats, rye, buckwheat, barley, sorghum, millet, or spelt.

2. *Meal* is the sound product made by grinding grain.

3. *Flour* is the fine, sound product made by bolting wheat meal and contains not more than 13.5 per cent. of moisture, not less than 1.25 per cent. of nitrogen, not more than 1.0 per cent. of ash, and not more than 0.50 per cent. of fiber.

4. *Graham flour* is unbolted wheat meal.

5. "*Whole wheat flour*," "*entire wheat flour*," improperly so called, is fine wheat meal from which a part of the bran has been removed.

6. *Gluten flour* is the product made from flour by the removal of starch and contains not less than 5.6 per cent. of nitrogen and not more than 10 per cent. of moisture.

7. *Maize meal, corn meal* or *Indian corn meal* is meal made from sound maize grain and contains not more than 14 per cent. of moisture, not less than 1.12 per cent. of nitrogen, and not more than 1.6 per cent of ash.

8. *Rice* is the hulled and polished grain of *Oryza sativa*.

9. *Oatmeal* is meal made from hulled oats, and contains not more

* Circular No. 13, Office of the Secretary, U. S. Dept. Agriculture.

than 8 per cent. of moisture, nor more than 1.5 per cent. of crude fiber, not less than 2.24 per cent. of nitrogen, and not more than 2.2 per cent. of ash.

10. *Rye flour* is the fine sound product made by bolting rye meal and contains not more than 13.5 per cent. of moisture, not less than 1.36 per cent. of nitrogen, and not more than 1.25 per cent. of ash.

11. *Buckwheat flour* is bolted buckwheat meal and contains not more than 12 per cent. of moisture, not less than 1.28 per cent. of nitrogen, and not more than 1.75 per cent. of ash.

Where percentages are given in these standards they are, of course, intended to represent normal limits rather than averages or extreme limits.

*Composition of Entire Grains.*

Wiley* estimates the approximate composition of average typical American grains as follows:

PERCENTAGE COMPOSITION OF ENTIRE GRAINS (WILEY).

| | Barley | Buck-wheat. | Maize. | Oats. | Rice unhulled. | Rye | Wheat. |
|---|---|---|---|---|---|---|---|
| Moisture | 10.85 | 12.00 | 10.75 | 10.00 | 10.50 | 10.50 | 10.60 |
| Protein (Nitrogen × 6.25) | 11.00 | 10.75 | 10.00 | 12.00 | 7.50 | 12.25 | 12.25 |
| Fat (Ether Extract) | 2.25 | 2.00 | 4.25 | 4.50 | 1.60 | 1.50 | 1.75 |
| Crude Fiber | 3.85 | 10.75 | 1.75 | 12.00 | 9.00 | 2.10 | 2.40 |
| Ash | 2.50 | 1.75 | 1.50 | 3.50 | 4.00 | 1.90 | 1.75 |
| Carbohydrates (diff.) | 69.45 | 62.75 | 71.75 | 58.00 | 67.40 | 71.75 | 71.25 |

*Composition of Mill Products.*

An extended study of the mill products of wheat made by Teller at the Arkansas Experiment Station, 1894 to 1898,† included a milling experiment in which the principal products of a long process (7 break) roller mill were analyzed with the following results:

PERCENTAGE COMPOSITION OF MILL PRODUCTS OF WHEAT (TELLER).

| | Patent Flour. | Straight Flour. | Low Grade Flour. | Ship Stuff. | Bran. | Whole Wheat. | Pure Germ. |
|---|---|---|---|---|---|---|---|
| Moisture | 13.75 | 13.90 | 13.22 | 12.25 | 12.85 | 13.90 | 6.80 |
| Ash | .33 | .47 | .90 | 3.12 | 5.80 | 2.15 | 4.65 |
| Crude Fiber | .17 | .26 | .74 | 3.55 | 6.14 | 2.17 | 1.60 |
| Fat | 1.05 | 1.25 | 1.70 | 4.80 | 5.20 | 2.15 | 14.38 |
| Protein (Nitrogen × 5.7) | 9.69 | 10.37 | 12.88 | 16.36 | 15.56 | 12.31 | 36.00 |
| Carbohydrates (diff.) | 75.01 | 73.75 | 70.56 | 59.02 | 54.45 | 63.32 | 36.55 |
| Total Nitrogen | 1.70 | 1.82 | 2.26 | 2.87 | 2.73 | 2.16 | 6.34 |
| Proteid Nitrogen | 1.65 | 1.72 | 2.20 | 2.68 | 2.51 | 1.98 | |
| Amid Nitrogen | .05 | .10 | .06 | .19 | .22 | .18 | ‡ |

* Bull. 45, Bur. Chem. U. S. Dept. Agriculture.

† Buls. 42 and 53, Ark. Expt. Station (Fayetteville, Ark.).

‡ The germ is richer in amid nitrogen than other parts of the wheat kernel.

Under the system of milling now practiced in the Northwest a number of "streams" of flour are produced which are afterward united in different proportions to form the market grades of flour. Snyder has recently published* analyses of the different "streams" as obtained in milling No. 1 Northern wheat by typical modern machinery. A sample of wheat containing 13.07 per cent. moisture and 2.28 per cent. nitrogen gave streams of flour containing from 9.01 to 11.68 per cent. moisture, and from 1.92 to 2.95 per cent. of nitrogen. The "gliadin number," or percentage of the total nitrogen existing in the form of alcohol-soluble proteids, varied from 39.51 to 66.67. It is interesting to note that some of the streams of flour thus obtained from average wheat in the ordinary milling process contain considerably more nitrogen than was recently found in the majority of so-called gluten and diabetic flours obtained in the market.†

For additional analyses and results of experiments upon the digestibility and nutritive value of cereal products see Bul. 13, Part 9, Bureau of Chemistry, and Buls. 28, 67, 85, 101, 126, and 143, Office of Experiment Stations, U. S. Department of Agriculture.

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* Bul. 85, Minn. Expt. Station, St. Anthony Park, Minn., 1904.

† Sherman and Burr: *New York Medical Journal*, 74, 686 (Oct. 12, 1901).

## CHAPTER XIV.

### Milk.

Cows' milk is concisely described as essentially an aqueous solution of milk sugar, albumin, and certain salts, holding in suspension globules of fat and in a state of semi-solution casein together with mineral matter (Richmond). Small amounts of other compounds are also present, but need not be considered here.

Standard milk (whole milk) is defined * as the lacteal secretion obtained by the complete milking of one or more healthy cows properly kept and fed, excluding that obtained within fifteen days before and five days after calving, and contains not less than 12 per cent. of total solids, not less than 8.5 per cent. of solids not fat, and not less than 3.25 per cent. of milk fat.

These limits are considerably below the average and considerably above the lowest authentic figures which have been found. Average milk may be assumed to contain 12.9 to 13 per cent. of total solids made up of:—

| | Fat. | Protein. | Milk Sugar.† | Ash. |
|---|---|---|---|---|
| In round numbers ‡ ........................ | 4.0 | 3.3 | 5.0 | 0.7 |
| Estimated average ........................ | 4.00 | 3.35 | 4.88 | 0.72 |

The protein content of average milk is, therefore, about one-fourth of the total solids. In general the same relation holds in milk which is richer than the average. Each increase of 1 per cent. in total solids thus involves on the average an increase of 0.25 per cent. of proteids, the remaining 0.75 per cent. being practically all fat. This increase in proteids and fat is usually accompanied by a slight increase in ash and decrease in milk sugar. The following average percentages illustrate these relations in rich milk:—

| | Total Solids. | Fat. | Protein. | Milk Sugar. | Ash. |
|---|---|---|---|---|---|
| Average for 5 years; mixed evening milk of 400 to 500 cows.................... | 14.62 | 5.39 | 3.66 | 4.82 | 0.75 |
| Average of 13 unusually rich samples from individual cows.......................... | 18.03 | 7.76 | 4.68 | 4.76 | 0.83 |

* Circular No. 13, Office of the Secretary, U. S. Dept. Agriculture.

† The figures for milk sugar include the small amount of undetermined non-nitrogenous matter.

‡ These are the figures used in most publications of the U. S. Dept. Agriculture.

The composition of milk of less than average richness cannot be so definitely stated. In some cases there is a deficiency of fat and protein with no decrease in milk sugar, while in other cases the reverse is true. Usually if an unadulterated milk is poor in fat it will be found proportionately poor in protein, while if the fat is normal the protein is usually also normal and the low percentage of solids not fat is due to a deficiency in milk sugar.

Several hundred American analyses, made before 1890, compiled and averaged in ten groups arranged according to percentage of total solids, gave the following results.*

COOKE'S COMPILATION OF AMERICAN ANALYSES OF MILK.

| Group No. | Total Solids. Per Cent. | Fat. Per Cent | Proteids. Per Cent. | Sugar and Ash. Per Cent. | Group No. | Total Solids. Per Cent. | Fat. Per Cent. | Proteids. Per Cent. | Sugar and Ash. Per Cent. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 11.35 | 3.20 | 2.99 | 5.16 | 6 | 13.71 | 4.46 | 3.48 | 5.77 |
| 2 | 11.77 | 3.36 | 3.03 | 5.38 | 7 | 14.25 | 4.87 | 3.65 | 5.73 |
| 3 | 12.21 | 3.60 | 3.10 | 5.51 | 8 | 14.77 | 5.20 | 3.87 | 5.70 |
| 4 | 12.75 | 3.82 | 3.29 | 5.64 | 9 | 15.17 | 5.47 | 4.07 | 5.63 |
| 5 | 13.17 | 4.09 | 3.40 | 5.68 | 10 | 15.83 | 5.88 | 4.26 | 5.69 |

As a rule the percentage of milk sugar and ash is most nearly constant, that of fat is most variable, while the protein varies with the fat, but to a much smaller extent. The variations which may be regarded as usual and the extreme variations which the writer has found authentically recorded are as follows:—

| | Fat. Per Cent. | Solids Not Fat. Per Cent. | Proteids. Per Cent | Milk Sugar. Per Cent. | Ash. Per Cent |
|---|---|---|---|---|---|
| Usual variations......... | 3–6 | 8.5–9.5 | 3–4 | 4.6–5 | 0.7–0.78 |
| Extreme variations†.. | 1.04–14.67 | 4.90–13.76 | 2.86–9.98 | 2.33–5.28 | 0.66–1.44 |

The extreme variations are of no practical value as a means of determining the limits within which milk shall be considered unadulterated, partly because it is possible to practice "adulteration through the cow" (*i. e.*, by selection, feeding, and manner of milking to obtain "genuine" milk much below the normal quality), but mainly because the milk which reaches market is practically always the mixed product of several cows so that individual variations have comparatively little effect.

There are many causes of variation in the composition of cow's milk. Only the most important can be given here. Other con-

* Cooke: Vermont Agricultural Experiment Station Report for 1890, p. 97.

† Including only results obtained from apparently healthy cows, believed to have been milked regularly under normal conditions.

ditions being normal the percentages of fat and proteids are higher in autumn and winter than in spring and summer; they also increase as the amount of milk decreases toward the end of each period of lactation. Milk drawn in the evening is generally 0.3 to 0.4 per cent. richer in fat than that obtained in the morning, and at any one milking the last portions drawn are much richer than the first. The influence of a change of food upon the percentage composition of milk is usually only temporary. In general the peculiarities of breed* and the qualities of individual animals are the most important factors in determining the richness of milk. Aside from all these conditions the milk of individual cows is subject to considerable fluctuation, especially in fat content. Thus the analyses of 60 monthly samples of the mixed milk of about 500 cows showed a variation of 0.89 in the percentage of fat, the greatest deviation from the average being 0.50 per cent. About one-half of the determinations of fat in the milk of individual cows of the herd during the same period were more than 0.50 per cent. and about one-fifth were more than 1.0 per cent. above or below the average. Milk representing the mixed product of several farms, such as is now commonly sold in large cities, should, therefore, be much more uniform in composition than that of a single cow or a small herd.†

## TAKING AND PRESERVATION OF SAMPLES.

If the lot of milk to be sampled is small it can be mixed by pouring from one vessel to another from two to ten times, according to the extent to which the cream has separated, and the portion for analysis dipped, or withdrawn by means of a pipette, from near the center. When the sample is too large to be handled in this way it should be transferred if necessary to a cylindrical can and sampled by means of a Scovell tube.‡ In order to obtain a

* For comparison of the milk of different breeds see Richmond's Dairy Chemistry, pp. 122–126, Report of the New York State Expt. Station for 1891 (abstracted in the Expt. Station Record, 4, 263), and Report of the Wisconsin Expt. Station for 1901, p. 85.

† Fuller discussions of the variations in the milk of individual cows and mixed milk of herds will be found in some of the reference books given at the end of the chapter, in the papers on the composition of milk published annually by Richmond in the *Analyst*, and in Hittcher's Gesammtbericht über die Untersuchung der Milch, Berlin, 1899.

‡ Wiley's Agricultural Analysis, Vol. III., p. 470, Leach's Food Inspection and Analysis, p. 95. These sampling tubes are sold by dealers in dairy apparatus.

proper sample of a large lot of milk delivered in cans of the same diameter, it is only necessary to sample each can with the Scovell tube and mix the portions thus obtained.

The sample should be placed at once in a clean, dry, sterile bottle, tightly stoppered, and analyzed as soon as possible. Before withdrawing each portion for analysis the sample must be thoroughly mixed by pouring — not by shaking.

If the analysis cannot be made at once or if the sample is to be kept for some time after the analysis, it must either be stored at a temperature near the freezing point or preserved by the addition of an antiseptic. Formaldehyde * added, while the milk is still fresh, in the proportion of 1 : 1000 will preserve the sample for months without apparent change. This amount of formaldehyde has a scarcely perceptible influence upon the analytical results. If preservation for only a few days is required a smaller amount of formaldehyde should be used, 1 : 2000 to 1 : 10,000 according to the freshness of the milk.

## PRELIMINARY OR PARTIAL EXAMINATION.

### Determination of Specific Gravity.

The specific gravity of milk is usually between 1.029 and 1.034. Since cream is considerably lighter than milk, the specific gravity would be lowered by the addition of water or of cream, but cases in which genuine milk shows a low specific gravity as a result of high fat content are very rare. As already explained, high percentages of fat are normally accompanied by high percentages of proteids, so that in most cases the specific gravity is higher in rich than in poor milk. With practice the samples which are exceptions to this rule can usually be detected by noticing the apparent viscosity and opacity of the milk as it runs from the surface of the lower bulb of the lactometer. The specific gravity taken in connection with this appearance is much used as a preliminary test by milk inspectors and is recommended by Richmond as the best means of rapidly testing each lot of milk received by a large dairy.

The Quevene, Veith, and Soxhlet lactometers are hydrometers of sufficient range for use with milk and so graduated as to read the "excess gravity" over water taken as 1000. Thus a milk of

* Other preservatives are sometimes useful. See Richmond's Dairy Chemistry, p. 144.

1.0315 specific gravity gives a lactometer reading of 31.5°. These instruments are often made to include a Fahrenheit thermometer, the scale of the latter being on the same stem with the lactometer scale. The lactometer reading should be taken between 50° and 65° F. and corrected for temperature by adding or subtracting 0.1° for each degree F. above or below 60. The New York Board of Health lactometer has a scale reading zero in pure water and 100 at 1.029 specific gravity. To convert the readings of this scale into the lactometer degrees described above, multiply by 0.29.

## Volumetric Determination of Fat.

Babcock, in 1890, introduced the first satisfactory rapid method for the determination of fat in milk. On mixing milk with approximately an equal volume of strong sulphuric acid, the casein is dissolved while the fat remains unchanged and can be separated by centrifugal force. The test is performed in a bottle with a neck so graduated that the percentage of fat can be read off directly upon removing the bottle from the centrifuge.

*Determination.* — Measure 17.6 c.c. of milk at 14° to 18° (about 55° to 65° F.) and introduce into the test bottle. Add 17.5 c.c. of sulphuric acid of 1.82 sp. gr. (commercial concentrated acid is usually the right strength) allowing the acid to flow down the side of the bottle so as not to mix with the milk. When acid has been added to all of the bottles and everything is ready to start the whirling, mix the milk and acid quickly and thoroughly by shaking and continue the shaking until the curd is in solution and the liquid has reached a permanent and uniform color. Then place an even number of the bottles in opposite pockets in the machine and whirl at the rate of 800 to 1200 revolutions per minute, according to the diameter of the wheel which carries the bottle. After whirling five minutes, fill with hot water to the shoulder of the bottle and whirl two minutes, then fill with hot water to near the top of the graduation in the neck and whirl again for two minutes. The percentage of fat is now shown by the height of the column in the graduated neck of the bottle.

*Notes.* — The capacity of the graduated neck of the bottle from 0 to 10 is 2 c.c. It is assumed that the 17.6 c.c. of milk taken for the determination will weigh ten times as much as 2 c.c. of warm butter fat. It is important that the final readings be taken while the fat is still warm. On account of the unavoidable contraction

of the fat while taking these readings it is customary to read from the bottom of the lower to the top of the upper meniscus. The result is usually within 0.2 per cent. of that found by the gravimetric method. The column of fat should be of a clear yellow color throughout. If the acid used is too weak, flocks of undissolved casein are apt to be found in the lower part of the fat column; if too strong the acid may char the fat. For a full discussion of the details of the test with directions for applying it to other dairy products see the work of Farrington and Woll.*

The most important modifications of the Babcock method are fully described in Richmond's Dairy Chemistry, pp. 174–192.

### Calculation of Solids from Specific Gravity and Fat.

Many formulæ have been proposed by which to calculate the percentage of solids in milk from the percentage of fat and the specific gravity. The results thus obtained are sufficiently accurate for many technical purposes and often for routine inspection work which is not to be made the basis of legal action. Such formulæ may be found in many of the works referred to at the end of this chapter. They are necessarily based on the assumption that each per cent. of fat causes a definite decrease, and each per cent. of solids not fat a definite increase, in the specific gravity.

Since the solution densities of proteids, milk sugar, and milk ash differ considerably † any change in the relative proportions of these constituents must alter the solution density of the solids not fat and thus diminish the accuracy of this method.

One of the simplest of these formulæ is that of Richmond:

$$\text{Total solids} = \frac{\text{Lactometer reading}}{4} + 1.2 \text{ fat} + 0.14.$$

With samples which do not differ greatly from the average composition the results thus calculated are usually accurate within 0.25 per cent.

## DETERMINATION OF FAT, PROTEIDS, MILK SUGAR, AND ASH.

### Total Solids and Ash.

Into an accurately weighed flat-bottomed platinum dish introduce two to five grams (depending upon the size of the dish; see

* Testing Milk and Its Products. Madison, Wisconsin, 1899.

† Allen: Commercial Organic Analysis, Vol. IV., p. 166.

below) of the thoroughly mixed milk and weigh quickly to the nearest milligram. If this weighing cannot be accomplished within one minute the dish should be covered with a weighed watch glass to retard evaporation. Place the open dish on a water bath or on top of the boiling water oven until nearly all of the water is expelled; dry to constant weight in a boiling water oven or an air bath kept constantly at 97° to 100°. The residue is somewhat hydroscopic and must be weighed quickly upon removal from the desiccator in order to obtain the correct amount of total solids.

Ignite the dry solids in a muffle at 550° to 600°, or, if this is not feasible, regulate a Bunsen burner to give a very small colorless flame and apply this carefully with constant attention so that no part of the dish is heated above the lowest possible redness. The ash should be white or very light gray. After obtaining the weight of the ash it may be used in testing for preservatives, as described below.

*Notes on Total Solids.* — When the same portion is not to be used for the determination of ash a platinum dish is not essential. Lead foil bottle caps are then very convenient, as they are easily numbered by scratching, quickly heated and cooled, and so cheap that each dish can be rejected after being used once. In order that a larger surface may be exposed, the dish may contain dry sand or other porous material and a small stirring rod, weighed with the dish and used to stir the residue while drying. Unless absorbed upon porous material, no more than 0.5 gram of milk for each square centimeter of the area of the bottom of the dish should be taken for the determination.

The methods used in the Government Laboratory, London, for the determination of solids in sour or fermented milk and the estimation of the solids lost in fermentation have recently been described by Thorpe.*

*Notes on Ash.* — Normally about two-thirds of the ash of cows' milk is insoluble in hot water. The presence of a larger proportion of soluble ash may be due to the use of mineral preservatives or to the addition of salts to restore the density and ash content to milk which has been watered. Special tests for some of the mineral preservatives are given beyond. In important cases it may be necessary to analyze the ash to show whether it is of normal char-

* *Journ. Chem. Soc.*, 1905, 87, 206.

acter. Richmond * gives the following as the average composition of milk ash: Calcium oxide, 20.27 per cent.; magnesium oxide, 2.80 per cent.; potassium oxide, 28.71 per cent.; sodium oxide, 6.67 per cent.; phosphoric anhydride, 29.33 per cent.; chlorine, 14.00 per cent.; carbonic anhydride, 0.97 per eent.; sulphuric anhydride, trace; ferric oxide, etc., 0.40 per cent. According to most other writers † milk ash contains sulphates, but only traces of carbonates. A sample of ash from the mixed milk of about 500 cows which has been partially examined by Thompson and the writer, shows no appreciable amount of carbonates and only traces of sulphates. The ash was prepared at known temperatures with the precautions given in Chapter I. According to the results thus far obtained there was no volatilization of chlorides below 650°, but even at 450° to 500° there was considerable loss of chlorine due doubtless to the formation of acid products in the combustion of the organic constituents of the milk.

### FAT. — GRAVIMETRIC DETERMINATION.

*Adams' Paper Coil Method.*

In this method the milk is dried on porous paper, the fat extracted by means of ether into a weighed flask, the ether evaporated, and the fat weighed.

*Apparatus.* — (1) Strips of thick absorbent fat-free paper about 55 cm. long and 6.25 cm. wide,‡ each rolled into a loose coil and fastened by means of a piece of wire or fat-free thread. If difficulty is found in making a loose coil, two pieces of fat-free string may be laid lengthwise upon the paper strip before rolling it up. This, however, should not be necessary.

(2) A Soxhlet apparatus for ether extraction; the form having ground glass connections being recommended.

(3) A safety water bath or, preferably, an electric heater which can be easily regulated.

*Determination.* — Mix the milk thoroughly and absorb a known amount, about 5 grams, on the paper coil. The milk can be measured by means of a 5 c.c. pipette and delivered directly upon the

* Dairy Chemistry, p. 32.

† See tabulated analyses in Stohmann's Milch- und Molkereiproducte, p. 89.

‡ Strips of paper especially prepared for this purpose are made by Schleicher and Schüll. If these are not available the paper strips must be very carefully extracted before use. See Richmond's Dairy Chemistry, pp. 91–93.

coil, but as milk is more viscous than water an ordinary 5 c.c. pipette will deliver less than 5 c.c. of milk, so that this method can be made accurate only by determining experimentally the amount of milk which the pipette actually delivers. A better method is to pour about 5 c.c. into a very small beaker, weigh quickly to centigrams and at once absorb the milk by standing the coil in the beaker. The absorption can be hastened by inclining the beaker and rotating the coil. The last drops in the beaker must be carefully absorbed. Stand the coil upon the dry end and re-weigh the beaker quickly to centigrams. If carried out rapidly this method is considerably more accurate than measuring with a pipette. Dry the coil thoroughly in a boiling water oven, place in a Soxhlet extractor, and extract with anhydrous ether, using the electric heater or safety water bath and keeping the apparatus as far as possible from free flames. If not more than 5 grams of milk is used and the extractor siphons at intervals of 10 to 15 minutes, the extraction need not be continued longer than three hours. At the end of the extraction disconnect the apparatus, remove the coil, replace the extractor, recover nearly all the ether by allowing it to collect in the space formerly occupied by the coil, return the ether to its bottle, and heat the flask containing the fat in a boiling water oven until the weight is practically constant.

*Notes.* — As the milk is absorbed by the paper the greater part of the fat is left on or near the surface, so that it is very rapidly extracted by the ether. The coil must be thoroughly dried before extracting with ether. The drying can be hastened by pressing in the dry end of the coil so that the inner layers of the wet end are made to project in the form of a cone. Such a coil will usually be dry after standing two to three hours in the boiling water oven. On removing from the oven press back the projecting end of the coil and place it, milk end down, in the extractor; connect with the flask, pour in ether until it siphons into the flask, then enough more ether to cover about half of the coil. This is sufficient to avoid any danger of the flask going dry during the extraction, if the heat is so regulated that no perceptible amount of ether escapes through the reflux condenser. To dry the extract leave it in the boiling water oven for three hours, allow to cool for one-half hour, weigh, and then repeat, heating about one hour each time, until two successive weighings show a loss of less than one milligram. In laboratories where many determinations are made it is cus-

tomary to dry the extract for a fixed length of time (usually five hours), which has been found by experience to be sufficient.

*Babcock Asbestos Method.**

Provide a hollow cylinder of perforated sheet metal, 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disk of the same material. The perforations should be about 0.7 mm. in diameter and about 0.7 mm. apart. Fill loosely with from 1.5 to 2.5 grams of freshly ignited, woolly asbestos, free from fine and brittle material, cool in a desiccator, and weigh. Introduce a weighed quantity of milk (between 3 and 5 grams) and dry at 100° to constant weight. This weight shows the percentage of total solids. Place the cylinder in an extractor and complete the determination of fat as described above.

This method avoids the possibility of having any ether-soluble matter in the porous substance used to absorb the milk. It is especially recommended for the determination of fat in cream which cannot be absorbed upon the paper coil without previous dilution.

PROTEIDS.

Formerly milk proteids were precipitated, or the milk evaporated to dryness, and the residue after washing with ether and dilute alcohol was dried, weighed, burned, and the ash deducted. On account of the difficulty of completely removing the sugar and fat, the results thus obtained were usually too high; so that in the older statements of the composition of milk (some of which are still often quoted) the proteids were usually overestimated.

Protein in milk is now calculated from the nitrogen content multiplying the latter by the usual factor 6.25 or sometimes by a special factor, 6.33 or 6.37, based on analyses of milk proteids showing less than 16 per cent. of nitrogen.

To determine the total nitrogen in milk pour 5 to 10 grams of the sample into a small beaker, weigh quickly to centigrams, pour the milk carefully into a Kjeldahl flask, re-weigh the beaker, and introduce 20 to 25 c.c. of concentrated sulphuric acid into the flask in such a way as to wash down any milk which may have remained in the neck. Add 0.7 gram of mercury, heat gently over a very small flame until most of the water is expelled and no more frothing or spirting occurs, then increase the size of the flame and complete the determination as described in Chapter II.

* Bul. 46, Bur. Chem., U. S. Dept. Agriculture.

Casein can be precipitated by acidulating the milk or by means of magnesium sulphate. Determination of nitrogen in the washed precipitate shows the amount of casein in the milk. Albumin can be precipitated by boiling the filtrate and determined in the same manner. Detailed directions for these determinations will be found in Bulletin 46, Bureau of Chemistry, U. S. Dept. Agriculture.

## MILK SUGAR OR LACTOSE.

In most cases the direct determination of lactose is unnecessary as the difference between the percentage of total solids and the sum of the percentages of fat, proteids, and ash should not differ from the true percentage of lactose by more than 0.1 to 0.2 per cent. When direct determination is desired either the polariscopic method or one of the methods based upon the reduction of copper can be used. In the former case the proteids are precipitated and the solution clarified by means of mercuric nitrate or iodide, in the latter by cupric hydroxide or acetic acid, alum, and aluminum hydroxide.

### *Optical Determination.**

Place 65.8 grams of milk in each of two flasks, one graduated at 100, the other at 200 c.c., to each add 4 c.c. of mercuric nitrate solution,† fill to the mark, shake, filter through dry paper, and polarize in a 200 mm. tube in the Schmidt and Haensch polariscope.

In each case the reading is too high on account of the volume occupied by the precipitate which contains the proteids and fat of the milk. This volume is twice as great in proportion in the 100 c.c. as in the 200 c.c. flask. The corrected reading and the volume occupied by the precipitate can, therefore, be calculated by the method of double dilution as in the following example: —‡

Weight of milk taken 65.8 grams, or twice the "lactose normal" weight § for the Ventzke scale.

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* Wiley and Ewell: *Journ. Amer. Chem. Soc.*, 1896, 18, 428.

† To prepare this solution dissolve mercury in twice its volume of nitric acid, 1.42 specific gravity, and add to the solution an equal volume of water; or prepare a solution of equal strength by dissolving solid mercuric nitrate in water acidulated with nitric acid.

‡ Compare Wiley's Agricultural Analysis, Vol. III., pp. 102, 278.

§ Calculated from the sucrose normal weight and the approximate specific rotatory powers of sucrose and lactose. These data and directions for the manipulation of the polariscope have been given in Chapters V. and VI.

Average reading from 100 c.c. flask, 10.45.
" " " 200 c.c. " 5.075.

Then

$$10.45 - (5.075 \times 2) = 0.30 \text{ (half the error in the higher reading).}$$
$$10.45 - (0.30 \times 2) = 9.85 \text{ (corrected reading for 100 c.c. flask).}$$
$$9.85 \div 2 = 4.925, \text{ corrected percentage of lactose.}$$

The volume of the precipitate is calculated as follows:

$10.45 \div 2 = 5.225$, apparent percentage of lactose (100 c.c. flask).

Then

$$5.225 : 4.925 :: 100 : x.$$

$x = 94.26$, the volume of solution in the 100 c.c. flask. Hence the volume of the precipitate is 5.74 c.c.

*Determination by Fehling Solution.**

Dilute 25 c.c. of milk with 400 c.c. of water in a 500 c.c. flask, add 10 c.c. of the copper sulphate solution used in the Fehling method, mix and add 4.4 c.c. of normal sodium or potassium hydroxide (or an equivalent amount of a weaker standard solution), fill to the mark, mix, and filter through dry paper. The filtrate must contain copper in order to ensure the absence of any trace of free alkali. In this clear filtrate lactose can be determined by means of Fehling solution either by Defren's method as described in Chapter V., or by Soxhlet's method, Bul. 46, *l. c.* The milk is so greatly diluted in clarifying the solution that the volume of the precipitated proteids and fat can be neglected.

If lactose is to be determined volumetrically, the proteids can be precipitated and the solution clarified as described in Richards and Woodman's Air, Water, and Food.

INTERPRETATION OF RESULTS.

The principal adulterations affecting the percentages of nutrients in milk are the addition of water (sometimes containing dissolved solids) and the removal of cream. These adulterations are sometimes difficult to detect with certainty because genuine cows' milk varies considerably both in fat and in other solids. Since the percentage of fat is more variable than that of solids not fat, skimming is more difficult to detect than watering. If as much as one-fourth of the fat were removed, the skimming would usually be in-

* Bul. 46, *loc. cit.*

dicated by the disturbance of the normal relation between the percentage of fat and that of proteids or of solids not fat, but the analysis cannot be said to prove the removal of cream unless it shows a lower percentage of fat than is ever found in genuine normal milk. Starting with average milk containing 4 per cent. fat and 9 per cent. solids not fat, one-tenth of the fat could be removed by skimming and the resulting product containing 3.6 per cent. fat could not be distinguished by analysis from genuine milk, while if the fat were reduced to 3.6 per cent. by watering, the solids not fat would be reduced to 8.1 per cent. which is sufficiently below the normal to be detected without difficulty. Occasionally genuine milk contains even less than 8.0 per cent. of solids not fat (the deficiency in most of these cases falling mainly upon the milk sugar) so that the limit of 8.5 for solids not fat might indicate watering where none had been practiced. Such errors are avoided by taking account of the proteids and ash. Milk should contain not less than 8.5 per cent. of solids not fat, 3.0 per cent. of proteids, 0.7 per cent. of total ash, 0.5 per cent. of ash insoluble in hot water. These four determinations will usually suffice to show whether the milk is genuine or has been watered with or without the addition of soluble solids.

UNITED STATES AND STATE STANDARDS FOR MILK, 1905.

| | Total Solids, Per Cent. | Solids not Fat, Per Cent. | Fat, Per Cent. | | Total Solids, Per Cent. | Solids not Fat, Per Cent. | Fat, Per Cent. |
|---|---|---|---|---|---|---|---|
| U. S. Dept. Agric. | 12 | 8.5 | 3.25 | New York | 12 | ........ | 3 |
| District of Columbia | ........ | 9 | 3.5 | North Carolina | 12 | 8.5 | 3.25 |
| *Georgia* | ........ | 8.5 | 3.5 | North Dakota | 12 | ........ | 3 |
| Idaho | ........ | 8 | 3 | Ohio | 12 | ........ | 3 |
| Illinois | ........ | ........ | 3 | " May–June | 11.5 | ........ | ........ |
| *Indiana* | ........ | 9 | 3 | *Oregon* | 12 | 9 | 3 |
| Iowa | 12.5 | ........ | 3 | Pennsylvania | 12 | ........ | 3 |
| Kentucky | 12 | ........ | 3 | Porto Rico | 12 | ........ | 3 |
| Maine | 12 | ........ | 3 | Rhode Island | 12 | ........ | 2.5 |
| Maryland | 12.5 | ........ | 3.5 | South Carolina | ........ | 8.5 | 3 |
| Massachusetts | 13 | 9.3 | 3.7 | South Dakota | 13 | ........ | 3 |
| " Apr.–Sept. | 12 | 9 | 3 | Utah | 12.5 | ........ | 3 |
| Michigan | 12.5 | ........ | 3 | Vermont | 12.5 | 9.25 | ........ |
| Minnesota | 13 | ........ | 3.5 | " May–June | 12 | ........ | ........ |
| Montana | 11.5 | ........ | 3 | Washington | ........ | 8 | 3 |
| *New Hampshire* | 13 | 9.5 | 3.5 | Wisconsin | ........ | ........ | 3 |
| " Apr.–Sept. | 12 | ........ | 3 | Wyoming | 12 | ........ | 2.4 |
| New Jersey | 12 | ........ | ........ | " May–June | 11.5 | ........ | ........ |

*In most cases it is not necessary to show conclusively whether milk has been skimmed or watered, but only whether it meets the*

requirements of a legal or trade standard. The principal standards in force in the United States on April 1, 1905, are given in the accompanying table (p. 227) from Circular No. 74, Bureau of Animal Industry, U. S. Dept. Agriculture.

## PRESERVATIVES AND PRODUCTS OF FERMENTATION.

Only a few of the many products produced by the action of micro-organisms in milk and of the substances used to retard the formation of such products or to hide their presence can be considered in this chapter.*

### FORMALDEHYDE.

#### *Detection.*

Of the many methods available for the detection of formaldehyde in milk only three of the best known and most delicate will be given.

*Sulphuric Acid Test.*†—Dilute 2 to 3 c.c. of milk with an equal volume of water in a test tube, add carefully so as not to mix the layers from 3 to 5 c.c. of concentrated commercial sulphuric acid or pure acid to which a trace of ferric salt has been added. If formaldehyde is present a violet ring forms at the junction of the two liquids. The charring of the milk by the sulphuric acid, makes it difficult to define the delicacy of the test. One part of formaldehyde in 100,000 of milk can be detected if the milk is fresh and the test is applied soon after adding the preservative.

*Hydrochloric Acid Test.*‡—Mix 10 c.c. of milk and 10 c.c. of concentrated hydrochloric acid containing about 2 mgs. of ferric chloride and heat slowly nearly to boiling, rotating the mixture occasionally to ensure solution of the curd. In the presence of formaldehyde a violet color develops, otherwise the solution slowly turns brown. The test is best performed in a porcelain casserole and in case of doubt the violet color is made much more distinct by adding 50 to 75 c.c. of water after having heated just below boiling for about a minute. The liquid must be observed

* Trade names and analyses of many proprietary preservatives will be found in the Year-Book of the U. S. Department of Agriculture for 1900, Chapin's Theory and Practice of Infant Feeding, and the *Zeitschrift für die Untersuchung der Nahrungs- und Genussmittel.*

† Hehner: *Analyst*, 1896, 21, 95.

‡ Leach: Ann. Rpts. Mass. State Board of Health, 1897, 558; 1899, 699; Food Inspection and Analysis, p. 140. See also Chapter IV.

carefully at the moment of dilution as the color which is brought out in this way fades very rapidly. This test is delicate to 1 : 250,-000 but formaldehyde added to milk in such small quantities soon disappears. When added to the extent of 1 : 50,000 to 1 : 100,000 the presence of formaldehyde in the milk will be shown by this test for from 1 to 5 days.* In testing sour or stale milk the brown color noted above will often obscure the reaction given by a small amount of formaldehyde until the solution is diluted with water, but at this point the violet color can be seen even though the milk may have been much charred by the acid.

*Gallic Acid Test.*—This test has been described in Chapter V. To apply it to milk, acidulate 30 c.c. with 2 c.c. of normal sulphuric acid and distil. To the first 5 c.c. of distillate add 0.2 to 0.3 c.c. of a saturated solution of gallic acid in pure alcohol, incline the test tube and pour in slowly 3 to 5 c.c. of concentrated sulphuric acid. The presence of formaldehyde is shown by the characteristic blue ring described in detail in Chapter V. In the writer's experience this test is at least twice as delicate as either the sulphuric or the hydrochloric acid test. The latter would be sufficiently delicate for all practical purposes if milk samples could always be tested while fresh, but when small amounts of formaldehyde have been added one or two days previously, the gallic acid test may show the preservative where either of the other tests would fail. This gallic acid reaction may also be used very satisfactorily as a means of confirming any doubtful results obtained by either of the preceding tests as there is little danger of interference due to charring or to the appearance of other colors.

### *Determination.*

Very small amounts of formaldehyde in milk can be determined by the following method, which is essentially that of Smith,† except that a larger quantity of sample is used.

To 300 c.c. of milk in a round-bottomed flask of about one liter capacity add 3 c.c. of (1 : 3) sulphuric acid and some glass beads to prevent bumping, heat gradually to boiling, preferably by means of a small rose-top burner, and distil until the distillate measures 60 c.c. Transfer this to a 100 c.c. flask, add 10 c.c. of standard

* Rivas: *University of Pennsylvania Medical Bulletin*, 1904, 17, 175. Unpublished experiments by Williams and the writer.

† *Journ. Amer. Chem. Soc.*, 1903, 25, 1036.

potassium cyanide solution, approximately tenth-normal, and mix; add a mixture of 15 c.c. of tenth-normal silver nitrate and 6 to 8 drops of 50 per cent. nitric acid, fill to the mark, shake, and filter through dry paper. Determine the excess of silver by the Volhard method and calculate the results as explained in Chapter V. under the description of the cyanide method as used for commercial solutions of formaldehyde. The precautions and the directions for standardizing there given should also be noted.

By this method from 32 to 39 per cent. of the formaldehyde in the milk is recovered and determined in the distillate. Assuming that the amount recovered represents 35 per cent. of the quantity in the milk, the latter can be estimated with a probable error of about one-tenth. Using this as a means of studying the disappearance of formaldehyde in milk, it was found in a typical experiment in which the proportion added was 1:40,000, that nearly three-fourths of the preservative had disappeared after two days at room temperature. After four days no formaldehyde was shown by this method but the violet color on dilution with water after heating with hydrochloric acid containing ferric chloride was unmistakable. The latter reaction could still be obtained after the mixture had stood for two weeks. When formaldehyde is added to milk in large proportion, 1 : 1000 to 1 : 10,000 as in the preservation of samples for analysis or reference, the rate of disappearance is much slower.

## HYDROGEN PEROXIDE.

### *Detection.*

Hydrogen peroxide in uncooked milk is easily detected by adding to 10 to 15 c.c. of the milk, 2 to 3 drops of a 2 per cent. aqueous solution of paraphenylenediamine hydrochloride. In the presence of hydrogen peroxide a blue color appears either immediately upon shaking or after a few minutes depending upon the amount present. The reaction depends upon the action of an oxidizing enzyme in the milk and the condition of the milk, therefore, affects the delicacy of the test. According to Arnold and Mentzel * 1 part in 40,000 can be detected. Under ordinary conditions the delicacy is probably somewhat less than this. In comparative tests made immediately after adding the same amounts of

* *Ztschr. Unters. Nahr.-Genussm.*, 1903, 6, 306.

peroxide to sweet milks 1 to 2 days old and to very sour curdled milks 3 to 4 days old, the former were found to give the reaction much more strongly than the latter. In practice, however, the preservative would be added while the milk was sweet and would probably disappear entirely before the occurrence of curdling. Milk which has been boiled can be tested after adding an equal volume of fresh milk known to be free from peroxide.

*Determination.*

Chick,* in an investigation of the germicidal properties and rate of disappearance of hydrogen peroxide in milk, used a method based upon the titration of the iodine liberated by the peroxide on adding potassium iodide and sulphuric acid.

$$2KI + H_2O_2 + H_2SO_4 = K_2SO_4 + I_2 + H_2O.$$

Mettler† has used the method with satisfactory results in the following modified form: To 40 c.c. of water, 0.5 gram of potassium iodide, and 10 c.c. of 12 per cent. sulphuric acid in a glass-stoppered flask, add 10 c.c of the milk, stopper tight and allow to stand in a cool dark place for two and one-half hours. In order to guard against any possible loss of iodine during this time, use a flask with flaring mouth as described in connection with the determination of the iodine number (Chapter IX.), and fill the gutter around the stopper with a solution of potassium iodide. Finally titrate the iodine which has has been set free in the milk, using a fiftieth-normal solution of sodium thiosulphate. In this titration it is not necessary to use starch as indicator since the disappearance of the yellow color produced by the action of the iodine upon the proteids affords a satisfactory end point. Test analyses gave results about 3 per cent. too low, doubtless because of the absorption of iodine by the milk fat. This source of error can be avoided by curdling the milk with acid, filtering, and adding the iodide to a measured amount of filtrate; but this is considered inadvisable in view of the fact that the peroxide may be undergoing decomposition during the filtration.

## SALICYLIC ACID AND SALICYLATES.

Hahn,‡ in experiments upon some of the methods most commonly recommended for the extraction of salicylic acid from foods,

* *Centralblatt für Bacteriologie und Parasitenkunde*, II. Abth., 1901, 1, 705.

† Thesis for the degree of Bachelor of Science, Columbia University, 1905.

‡ Thesis for the degree of Bachelor of Science, Columbia University, 1905.

recovered only a small percentage of the amount known to be present in milk. Separation of salicylic acid by distillation with steam is extremely slow, and extraction by shaking with ether or chloroform after precipitating the proteids by mercuric nitrate, is usually very incomplete. Better results can be obtained by the following modification of Remont's method: Acidulate 20 c.c. of milk with sulphuric acid, shaking well to break up the curd; add 25 c.c. of ether, mix, and allow to stand. Draw off 5 c.c. of the ether layer, evaporate to dryness, boil the residue with 10 c.c. of 40 per cent. alcohol, cool, adjust the volume to 10 c.c., filter through dry paper if necessary to remove fat, and test 5 c.c. of the filtrate (representing 2 c.c. of milk) by adding 2 c.c. of 2 per cent. solution of ferric chloride. A purple or violet color is produced if salicylic acid is present, and approximately quantitative results can be obtained by diluting to 50 c.c. and matching with standard solutions, observing all the precautions ordinarily required in colorimetric work.

### Boric Acid and Borates.

In routine work * the ash obtained in the usual course of analysis is treated with two drops of dilute hydrochloric acid † and about a cubic centimeter of water. A strip of turmeric paper is then placed in the dish, allowed to soak for a minute, removed and allowed to dry in the air. A deep red color changing to green or blue when treated with dilute alkali shows the presence of boric acid. According to Leach this reaction is delicate to 1 part in 8000. The well-known flame test with methyl alcohol is less delicate but can be used in confirmation.

The turmeric test is very satisfactory for the detection of boric acid in the ash, but since a considerable loss of boron may occur in burning off the organic solids of milk, it is better in all important cases to take a separate portion for this test and make it strongly alkaline with sodium or calcium hydroxide before evaporating to dryness. All of the boric acid then remains in the ash and can be determined quantitatively by Thomson's method.‡

* Leach: Food Inspection and Analysis, p. 144.

† Care should be taken to note whether any effervescence occurs. See carbonates and bicarbonates beyond.

‡ Glasgow City Anal. Soc. Reports, 1895; *Analyst*, 1896, **21**, 64; Sutton's Volumetric Analysis, 8th Ed., p. 100, 9th Ed., p. 98; U. S. Dept. Agriculture, Bur. Chem., Bul. 65, p. 110. The standard alkali used in the final titration should be freshly standardized against boric acid.

## Fluorides.

If milk containing a small amount of fluoride is evaporated and the solids burned to ash in the usual way the fluorine is likely to be almost entirely lost. In the presence of a considerable excess of alkali this loss of fluorine does not occur.

To detect or determine fluorides, add 1 gram of sodium carbonate to 100 c.c. of milk, evaporate, and burn to ash. If only qualitative results are required, examine for fluorides by the well-known etching test on glass. For a quantitative determination of the fluorine, leach the mixture of ash and sodium carbonate thoroughly with hot water, nearly neutralize with sulphuric acid, leaving the solution slightly alkaline, and then apply Rose's method as modified by Treadwell and Koch: *Ztschr. anal. Chem.*, 1904, 43, 469.

## Carbonates and Bicarbonates.

When milk contains the equivalent of 0.05 per cent. of sodium carbonate the ash obtained by direct ignition of the solids shows effervescence on addition of hydrochloric acid. Such effervescence is rarely if ever seen in the ash of pure milk, but since Richmond has found small amounts of carbonic acid in the ash of milk believed to have been pure,* the presence of carbonate or bicarbonate should be confirmed by applying Schmidt's test, in which 10 c.c. of milk are mixed with an equal volume of alcohol and a few drops of a 1 per cent. solution of rosolic acid. The color is brownish yellow in pure milk but rose-red in milk containing carbonate or bicarbonate. A comparative test with pure milk should always be made. The reaction is nearly as delicate as the test for effervescence in the ash.

Carbonates and bicarbonates are not to be regarded as preservatives, but as adulterants added to hide the presence of acid and thus give the milk a fraudulent appearance of freshness.

## Acidity.

To determine the total acidity of milk, dilute 10 c.c. of sample with 90 c.c. of water and titrate with tenth-normal sodium or potassium hydroxide using phenolphthalein as indicator. The acidity is usually considered to be due mainly to lactic and car-

* The use of salicylates or other alkali salts of organic acids as preservatives may also give rise to carbonates in the ash.

bonic acids. In order to eliminate the carbonic acid, the mixture of milk and water may be boiled in a beaker loosely covered with a watch glass for ten minutes. Both the total acidity and the acidity after boiling are often calculated as due to lactic acid. The results are also frequently given as "degrees of acidity." This term unfortunately has no definite meaning when applied to milk and must be explained in each case. It is, therefore, recommended that results be expressed as "total acidity equivalent to per cent. of lactic acid" and "acidity after boiling equivalent to per cent. of lactic acid."

In a very recent paper * Thorpe recommends that the volatile acids be calculated as acetic acid and determined as follows: To 10 grams of milk in a platinum dish, add a little phenolphthalein and one-half the volume of tenth-normal sodium hydroxide which would be required to neutralize the total acidity. Evaporate to dryness on a water bath with frequent stirring, add 20 c.c. of boiling distilled water, stir thoroughly to disintegrate and detach the milk solids from the dish, and titrate with tenth-normal sodium hydroxide. The difference between the total volume of alkali used in this determination and the volume required to neutralize 10 grams of the original milk gives a measure of the volatile acids in the sample.

The causes and significance of the acidity of milk have been studied recently by Touchat,† Vieth and Siegfeld,‡ Richmond and Harrison, § Kirsten, || and Haune. ¶

AMMONIA.

The Boussingault-Shaffer method for the determination of ammonia in urine ** has been thoroughly studied by Berg †† with regard to its applicability to the much smaller amounts of ammonia which occur in milk. The following is an outline of the method as adapted to milk :—

* *Journ. Chem. Soc.*, 1905, 87, 220.

† *British Food Journ.*, 1899, 199; Abs. *Jour. Chem. Soc.*, 1900, 78, ii, 582.

‡ *Milch Ztg.*, 29, 593; Abs. *Jahr. Thierchem.*, 1900, 261.

§ *Analyst*, 1900, 25, 120.

|| *Ztschr. Unters. Nahr.-Genussm.*, 1902, 5, 97.

¶ *Milch Ztg.*, 1904, 33, 659, 679, 709, 725; Abs. *Experiment Station Record* 1905, 16, 595.

** Shaffer: *Amer. Journ. Physiol.*, 1903, 8, 330.

†† Thesis for the degree of Bachelor of Science, Columbia University, 1904.

Fifty c.c. of the sample are mixed, in a two-liter round-bottomed flask, with an equal volume of methyl alcohol, 10 grams of sodium chloride, and 0.5 gram of sodium carbonate. The latter liberates the ammonia from its salts, the salt diminishes the dissociation of the carbonate and thus prevents or retards the cleavage of ammonia from the nitrogenous organic compounds in the milk, and the alcohol also tends to prevent cleavage both by diminishing dissociation and by lowering the boiling point of the mixture. The flask is closed with a stopper having an inlet tube to provide for the admission of air and an outlet fitted with a distilling head and connected with two condensing cylinders (in series) containing together from 15 to 25 c.c. of twentieth-normal acid diluted with water to 40 c.c. The cylinders are connected with a suction pump. By this arrangement it is possible to distil the ammonia into the absorption cylinders completely within fifteen minutes at a temperature not above 60°.

Fresh milk of good quality yields less than 0.001 per cent. of ammonia when treated by this method. If the sodium chloride is omitted in the distillation a larger amount of ammonia is obtained. For convenience the ammonia thus easily split off from organic matter by dilute sodium carbonate at 55° to 60° is called "cleavage ammonia." Both the ammonia existing as such in the milk and the "cleavage ammonia" increase rapidly under conditions which favor the decomposition of proteids by bacteria or molds, and it is believed that these determinations will prove useful in the sanitary examination of milk. The methods and results are more fully discussed in a paper on the determination of ammonia in milk in the *Journal of the American Chemical Society* for February, 1905.

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## ADDITIONAL NOTES AND CORRECTIONS.

Page 3.—Note also Morse and Taylor's new method of ultimate analysis: *Amer. Chem. Journ.*, 1905, **33**, 591.

Page 6, line 22.—Read sometimes instead of some.

Page 10.—Additional references, Milbauer: Quantitative Stickstoffbestimmung in Hydrazonen und Osazonen nach der Methode von Kjeldahl, *Ztschr. Zuckerind, Böhmen*, **28**, 338; Abs. *Biochem. Central.*, 1905, 469. Flamand and Prager: Analyse von Verbindungen mit Stickstoff-Stickstoff-Bindung nach Kjeldahl-Methode, *Ber. deut. chem. Ges.*, 1905, **38**, 559.

Page 18.—A modified form of the sodium peroxide method is recommended by Neumann and Meinertz: *Ztschr. physiol. Chem.*, 1904, **43**, 37. See also Dubois: *Journ. Amer. Chem. Soc.*, 1905, **27**, 729.

Page 26, last paragraph.—Note: Addition of calcium carbonate suffices for most sweet wines, but samples containing much free acid should always be neutralized carefully, using litmus paper as indicator.

Page 35.—Additional reference on determination of fusel oil, Schidrowitz: *Analyst*, 1905, **30**, 190.

Page 41.—Additional reference, Suhr: Kritische Studien über die quantitative Bestimmung des Glycerins, *Archiv für Hygiene*, 1892, **41**, 305.

Page 65, line 25.—Read Maquenne instead of Macquenne.

Page 70.—For discussion of ferrous thiocyanate as indicator in the volumetric determination of reducing sugars see Ling and Rendle: *Analyst*, 1905, 30, 182.

Page 78.—The details of manipulation and factors for calculation of furfurol and pentosans should be modified according to Kröber: *Journ. Landw.*, 1900, **48**, 357; *Ztschr. angew. Chem.*, 1902, 477; U. S. Dept. Agr., Bur. Chem. Bul. 73, p. 173.

Page 111.—Add to references on detection of dextrose, Wood: Chemical and Microscopical Diagnosis, New York, 1905.

Page 164.—Add to references on detection of cottonseed oil, Tolman: *Journ. Amer. Chem. Soc.*, 1905, **27**, 589.

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